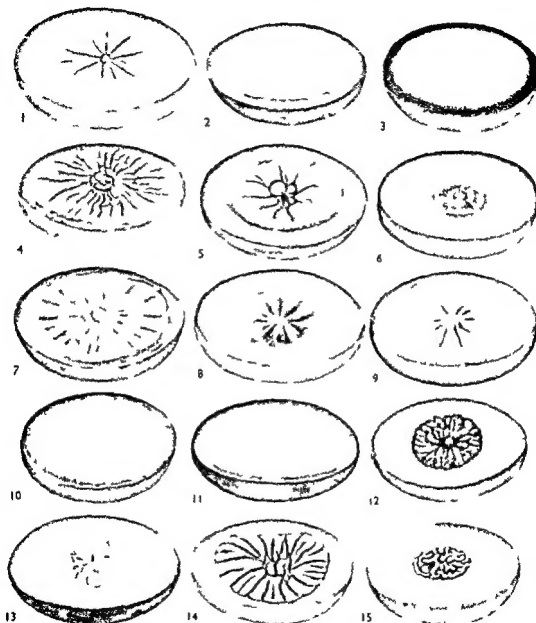


GIANT COLONIES OF RINGWORM FUNGI (Sabouraud's Maltose Agar)



(1) *Microsporum audouinii* (2) *M. canis* (3) *M. gypseum* (4) *Epidermophyton floccosum*
 (5) *Trichophyton tonsurans* (6) *T. sulphureum* (7) and (9) *T. purpureum* (8) *T. purpureum*
 (Glucose agar) (10) *T. mentagrophytes* Gypseum (11) *T. interdigitale* (12) *T. violaceum*
 (13) *T. schoenleini* (14) *T. ferrugineum* (15) *T. concentricum*

MEDICAL MYCOLOGY

INCLUDING
LABORATORY TECHNIQUE
AND
THERAPEUTIC RECIPES

By

N C DEY BSc MBBS (Cal) D Bact (Manchester) FDS (Lond)

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FOREWORD

By

Professor G PANJA MBBS (Cal) DB (Lond) FRC

Director of the School of Tropical Medicine Calcutta

WITH THIRTYEIGHT ILLUSTRATIONS THREE COLOURED
AND FORTY-SIX TEXT-FIGURES

CALCUTTA

1958

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**DEDICATED TO
THE MEMORY OF
MY WIFE**

FOREWORD

I have great pleasure in writing a foreword to the book on Medical Mycology by Professor N C Dey of the Assam Medical College. The author was a colleague of mine in the Calcutta School of Tropical Medicine for many years. [He devoted himself whole heartedly for several years in the study of Dermatology and isolation of pathogenic fungi from skin diseases and published a large number of original articles. Having thus gained considerable experience he has fully utilised his knowledge in the production of this book. The book has been amply illustrated with plates, text figures, coloured plates and diagrams collected and prepared during the long period of his work in the School as well as in the Assam Medical College. The speciality of the book is that the author has followed a natural classification of fungi pathogenic to man. The laboratory methods mentioned in the book are those which were found to be useful by the author while working in the School of Tropical Medicine, Calcutta.

I congratulate the author for sparing no pains in the production of such a valuable book. I am confident that his book will be a very useful guide to post graduate students and practitioners for whom it is meant.]

Sd/ G PANJA

Institute of the Department of Dermatology and
Professor of Bacteriology and Pathology
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Ti 30th December 1937
117 Vivekananda Road
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PREFACE

The author regrets very much for the various printing errors in the book specially with regard to the word coccidiomycosis in different pages. It should be read as coccidioidomycosis.

Little importance has been laid on the study of Medical Mycology in the undergraduate course of the Indian Universities. The subject has however been recently included as a part of the post graduate curricula of Dermatology and Tropical Medicine of the Calcutta University. This has been judiciously done as diseases caused by fungi are not uncommon in India a tropical country.

Since the time of the late Lt. Col. H. W. Acton then Director, The School of Tropical Medicine, Calcutta, 1932, I have had the opportunity of studying Medical Mycology as an Assistant Research Worker under him and others following him in the Department of Dermatology and Medical Mycology under the Indian Research Fund Association (present I. C. M. R.) and also in other places in India including the Assam Medical College, Dibrugarh as the Professor of Pathology. The articles on these works were published from time to time in Medical Journals in India and the present volume is the result of compilation of those articles. There are certain diseases like North and South American blastomycoses, coccidioidomycosis etc. which have not been authentically recorded in India and these diseases have been compiled from American literatures to make the book complete for the students and practitioners. In this volume common diseases caused by fungi in man and animals have been recorded. Common laboratory methods have been included in the appendix including certain recipes which might be of help to the medical practitioners. The author will consider his endeavour to be amply rewarded if the book becomes helpful to the students and practitioners in India.

The author is thankful to Sri H. C. Sarkar, Manager of Pressagents Private Limited, Calcutta, for the keen interest he has taken in the production of this volume.

Dibrugarh
the 15th December 1957

N C D

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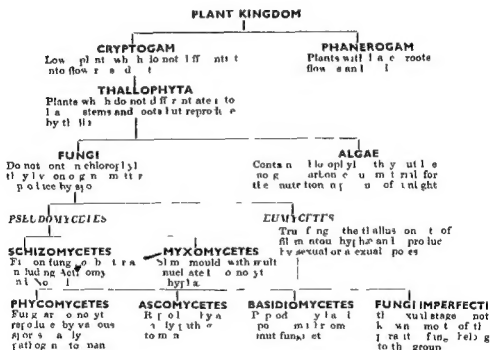
MEDICAL MYCOLOGY

CHAPTER I

INTRODUCTION

The science that deals with the study of fungi is called mycology. The position of fungi in the plant kingdom is shown in the following table.

TABLE I



Thallophyta are lower cryptogams which comprise two main groups : namely—Algae and Fungi : Algae are green thallophyta characterised by the presence of chlorophyll which is essential for the synthesis of carbohydrate from carbon dioxide in air in presence of sun light. They are therefore *autotrophic*. Fungi on the other hand are characterised by the absence of chlorophyll in their cells, they are *heterotrophic*. They are to depend for their growth on prepared carbonaceous food material from decaying organic matters as *saprophytes* or from the body of

other plants or animals as *parasites*. Fungi that grow on the surface of the body are called *ectoparasites* whereas those that grow in the tissue or internal organs are known as *endoparasites* causing systemic mycosis.

Schizomycetes (fission fungi) or bacteria are the lowest form of organisms in the vegetable kingdom and like fungi are devoid of chlorophyll. These are minute living units $1\ \mu$ or less in diameter. The question of the presence of nucleus in bacteria is still undecided. But the modern trend is to accept that bacteria contain nuclear chromatin which participates in the process of cell division. They do not however differentiate into any organ of reproduction although some of them reproduce by endospores.

Fumycetes (true fungi) are higher than bacteria but they show visible nuclear plant volutin and reproduce by sexual or asexual spores. Fungi resemble bacteria as they lack in chlorophyll but they differ from bacteria morphologically being larger in size than bacteria and show branching. Actinomycetes and Nocardi which are fungi resemble bacteria being $1\ \mu$ or less in diameter but unlike bacteria they show branching and the modern trend is to include the order Actinomycetales under the class Schizomycetes. (Bergey 1949)

DEFINITIONS

(*Fig. 1 to 10*)

Mycetes—A fungus

Mycosis—(pl. Mycoses). It is a disease caused by fungus.

Thallus—The entire vegetative apparatus of the fungus is called a thallus. It does not differentiate into roots, stems or leaves.

Hypha (pl. hyphae) (Fig. 1). It is a chain of cylindrical cells forming a filamentous structure.

Mycelium—(pl. mycelia) (Fig. 2). It is a felt like mass produced by a collection of intertwined hyphae collectively known as mycelium. Mycelia may be surface runners or they may be erect forming aerial hyphae which often bear spores (sporophores).

Coenocyte (Fig. 3). Multinucleated mass of protoplasm formed by division of nuclei but without any division of cytoplasm e.g. *Phycomycetes*.

Rhizoids (Fig. 4). Mycelium in certain cases is provided with root like structures called rhizoids e.g. *Rhizopus*.

Club (Fig. 5). The swollen portion of a hypha is called a club.

Racquets—(Racquet mycelia) (Fig. 6). These are racket shaped cells along the course of a hypha in which the clubbed end of one cell is attached to the thin end of the next cell. Mycelial racquets are often arranged in series.

Nodular Organs (Fig. 7). These are large rounded cells like knots formed by a few chlamydospores (See page 4) with radiating mycelia from the knot.

Pectinate bodies (Hyphae pectinate) (Fig. 8). These are swollen hyphae often curved and give off a row of abortive branches from one side roughly resembling a comb.

Spirals (Fig. 9). These are simple hyphae which develop into closely set spiral or coils. They simulate tendril like apparatus of higher plants.

Fig 1—Hypha



Fig 5—Clubs



Fig 6—Rocquets



Fig 2—Mycelium



Fig 3—Coenocyte



Fig 4—Rhizoids



Fig 7—Nodular organ



Fig 8—Pectinate body



Fig 9—Spiral

SEXUAL SPORES



Fig 10—Zygospore



Fig 11—Oospore



Fig 12—Ascocarp



Fig 13—Ascospores in asci



Fig 14—(a) Ascospore
(b) Hystium

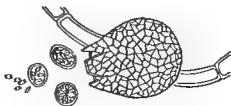


Fig 15—Perithecium



Fig 16
Basidiospores

To face page 2

Plate I

(Continued)

ASEXUAL SPORES



Fig 17
(a) Sporangiphore
(b) Sporangium



Fig 18
(a) Sporangiospore
(b) Columella



Fig 19—Blastospores



Fig 20—Arthrospore

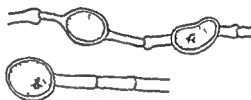


Fig 21
(a) Intercalary chlamydospore
(b) Terminal chlamydospore

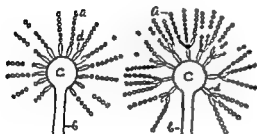


Fig 22
(a) Conidia vera (b) Conidiophore
(c) Columella (d) Sterigma

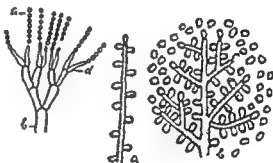


Fig 23—Macroconidia
(a) Thyse (b) Engrappe



Fig 24—Macroconidia
(a) Pointed (b) Cylindrical
(c) Blunt end



Fig 25—Phalide
with phalospore

Spores—Reproductive body of a cryptogam is called a spore. The spores may be sexual or asexual. Sexual spores are derived from the union of two gametes (Perfect stage) but asexual spores arise from fertile hyphae (Imperfect stage).

SEXUAL SPORES AND ACCESSORY STRUCTURES

Zygospore (Fig 10) It is a sexual spore resulting from fusion of two similar gametes (isogamous) as in zygomycetes.

Oospore (Fig 11) It is a sexual spore arising from fertilisation of a female cell by a male cell (two dissimilar gametes) antheridium and oogonium e.g. Oomycetes in Phycomycetes.

Ascocarp (Fig 12) Any fruit body containing asci is known as ascocarp.

Ascospores (Figs 13-14) These are sexual spores 4 or 8 in number enclosed in a membrane known as ascus (a cask or case). The ascus is usually cylindrical but in yeasts it is oval and is formed by fusion of the nuclei of two contiguous cells. The fusion is followed by two or three successive divisions resulting in 4 or 8 nuclei which subsequently form 4 or 8 spores by cutting out cytoplasm around the nuclei. Ascospores are characteristic of the class Ascomycetes.

Hymenium (Fig 14b) It is a tissue formed by a layer of spore producing structure found in the fruit bodies of Ascomycetes. The sterile hyphae interposed in the hymenium are known as paraphyses.

Perithecium (Fig 15) It is a globose fruit body containing organs of fructification interposed with fertile hyphae. A perithecium is enclosed in a loosely woven hymenium and shows asci and ascospores in it.

Apothecium When the fruit body of an Ascomycetes is wide open like a cup it is called apothecium e.g. *Dyscomycetes*.

Sclerotia These are composed of compact hard tissue formed by a close network of hyphae and capable of remaining dormant for a long period under unfavourable conditions. From this a new mycelium germinates under favourable conditions and bears an organ of reproduction e.g. sclerotia of *Claviceps purpurea*.

Basidiospores (Fig 16) These are sexual spores 4 in number held by peg like structures at the end of a club shaped sporebearing organ known as basidium (Pl. basidio). These are characteristic spores of the class Basidiomycetes.

ASEXUAL SPORES AND THE SPORE BEARING APPARATUS

Asexual spores may be endogenous e.g. sporangiospores or exogenous e.g. conidia.

Sporangiophore (Fig 17) The fertile hypha which bears a sporangium (Spore case) is called a sporangiophore.

Sporangiospore (Fig 18) These are asexual spores formed endogenously in a spore case called sporangium e.g. *Mucor*.

Columella—(small pillar) (Fig 18b) It is the terminal domeshaped swollen part of a sporangiophore bearing a sporangium.

The system of classification of spores by Saccardo is not mentioned here. It is preferable to follow the system of Vuillemin as modified here and mentioned below.

Thallospores Spores arising from thallus (vegetative part of a fungus) are called thallospores. These are subdivided into the following types:

(a) **Blastospores** (Fig 19) These are spores arising from the vegetative hyphae by a process of budding.

(b) **Arthrospores** (Fig 20) Mycelia when old show segmentations by transverse septa at close intervals. These segmented mycelia are either cubical or rounded and spores form in them without swelling of the mycelium.

(c) **Chlamydospores** These spores are formed by swelling of mycelial filaments. The process is associated with condensation of the protoplasmic substance, often at the cost of the neighbouring cells with formation of a thick cell wall with double contour. They may be intercalary along the course of the hypha (Fig 21a) or terminal at the end of a hypha (Fig 21b).

Conidiophore (Fig 22b) It is a fertile hypha bearing conidia.

Conidia There are several types of conidia. True conidia or *conidia vera* (Fig 22a) as contrast to thallospores are borne at the terminal part of a fertile hypha—a conidiophore. These are produced by abstriction of the fertile hyphae. The conidia may be bisipetal or acropetal. In the bisipetal type as in the case of *Penicillium* the oldest conidia are at the terminal part and become detached at the slightest disturbance. In the acropetal type the oldest cell is in the proximal part of the fertile hypha.

Aleuriospores (Microconidia) These are small spores about 3 to 4 μ in diameter borne on fertile hyphae. These are sessile. Unlike conidia they are liberated only after disintegration of mycelia. According to their arrangements they are said to form thyrse i.e. arranged laterally (Fig 23a) as in *Trichophyton rubrum* or en grappe (Fig 23b) as in *T. interdigitale* and *T. mentagrophytes*. These microconidia may be spherical, subspherical or ovoid.

Fuseaux (Macroconidia) These are spindle shaped large conidia either terminal or lateral. They are nonseptate when young but pluriseptate when old. They may be pointed, cylindrical or with blunt ends (Fig 24a, 24b, 24c). These are characteristic of dermatophytes.

Sterigma (Fig 22d) These are small stalks bearing conidia often in chains.

Phialide (Fig 2c) These are flask shaped stalks borne on sporophores and bear conidia.

Phialospores (Fig 25) Conidia borne on phialides are called phialospores.

Pycnidia These are minute globose or flask shaped fruit body with apical ostioles internally lined by conidiophores as in *Sphaeropsidales*. Conidia are discharged through the ostiole in the plan body which they parasitize.

Corremia These are stalked bodies of cylindrical shape formed by a cluster of conidiophores which run parallel in a fascicular form e.g. *Sulzbaceae*.

Sporothecium It is a sessile globose fruit body without stalks and contains a collection of conidiophores.

Acervuli These are fructifying bodies containing conidiophores packed together occurring in clusters or heaps and form a saucer like mass on the plant host e.g. *Melichonriales*

CLASSIFICATION

I Mycelium non septate coenocytic spores usually borne in a sporangium
Sexual fusion results in an oospore or a thick walled zygospore

Class PHYCOMYCETES

II Mycelium septate dimension of mycelia more than 1μ

A Reproduce by Ascospores which are sexual spores The sexual fusion results in formation of an ascus The ascus contains 4 or 8 ascospores

Class ASCOMYCETES

B Reproduce by basidiospore i.e. sexual fusion results in formation of a club shaped organ called basidium on the surface of which 4 basidiospores are borne by peglike structures attached to the basidium

Class BASIDIOMYCETES

C Reproduce by asexual spores sexual spores unknown

Class FUNGI IMPERFECTI

III Vegetative body 1μ or less in diameter and multiplies by binary fission

Class SCHIZOMYCETES

CLASS I PHYCOMYCETES

Phycomycetes They show nonseptate coenocytic vegetative mycelia and reproduce by both sexual and asexual spores They are divided into three subclasses

I **Archimycetes** These are primitive forms and found as parasites of water plants and animals but may sometimes cause diseases of land plants They mainly reproduce by motile flagellate spores called *oospores* In some cases they form oospores

II **Oomycetes** This subclass is characterised by formation of two dissimilar gametes in their sexual life The oogonium is fertilised either by a motile male sperm or by a specialised nonmotile male germ tube The oogonium or the female organ when ripe shows aggregation of protoplasm into one or more *oospheres* After fertilisation the oospheres round up to form *oospores* which often lie free in the oogonium (Fig 11)

III **Zygomycetes** The sexuality of this group of fungi is characterised by the formation of similar gametes and is called isogamous the process is known as conjugation and the product of union is called a *zygospore* (Fig 10) In the union the ends of two mycelia become swollen come in contact with each other and the terminal parts of the hyphae are segmented by a transverse wall The similar terminal cells are the conjugating cells (gametes) The walls between the conjugating cells disappear and the protoplasmic masses of both the cells unite forming

■ spore called zygospore. A zygospore has a double wall which is thick and rough. Under favourable conditions a germinating tube called a *promycelium* develops from a zygospore bearing a sporangium containing spores. This sporangium is similar to one found in the asexual spores. The classification of Phycomycetes is shown below.

SUBCLASSES OF PHYCOMYCETES

Mycelium absent	Subclass <i>ARCHIMYCETES</i>
Mycelium non-septate (coenocytic)	
A Sexual reproduction heterogamous—	Subclass <i>OOMYCETES</i>
B Sexual reproduction isogamous—	Subclass <i>ZYGOMYCETES</i>

CLASS II ASCOMYCETES

Ascomycetes. The vegetative body of Ascomycetes may be single celled (yeasts) or both yeastlike bodies and hyphae may be present. The hyphae are septate. Asexual spores or conidia are produced exogenously either from conidiophores or directly from hyphae (blastospores).

The sexual life in Ascomycetes is characterised by formation of specialised endogenous spores called ascospores borne on asci or sacs. This is the product of fusion of like or unlike sexual cells. The sexual process may not be hidden but naked during development e.g. true yeasts. But in other cases they may be protected and hidden in a specialised structure called perithecium. The class is divided into several subclasses as follows.

SUBCLASSES OF ASCOMYCETES

- 1 Asci produced from oogonium or by multiplication of diploid cell.
Ascogenous hyphae absent. Subclass *PROTOASCOMYCETES*
- 2 Asci borne on fertile ascogenous hyphae (Fusicomycetes)
 - A Asci in a closed perithecium Subclass *PLECTOMYCETES*
 - B Asci in a hymenium
 - (i) in an apothecium Subclass *DISCOMYCETES*
 - (ii) in a perithecium Subclass *PYRENOMYCETES*

CLASS III BASIDIOMYCETES

Basidiomycetes. The vegetative growths of these fungi show septate filamentous hyphae. They reproduce by basidiospores which are sexual spores. These are borne exogenously on special club-shaped organs called basidia. In a typical case a basidium bears four spores held by peg-like structures attached to the basidium. Mushrooms (toadstools) are fungi of this group. Lower fungi of this group are rust and smut fungi.

CLASS IV FUNGI IMPERFECTI

Fungi Imperfecti The fungi of this group do not show any sexual spore i.e. the life cycle is incomplete or better called incompletely known. The sexual life of these fungi has not been discovered and they are therefore called Fungi Imperfecti. In the words of Dodge "The Fungi Imperfecti are conceived as a dumping ground until the gaps in our knowledge have fulfilled." In the asexual life they appear similar to Ascomycetes and at least some of them have been found to produce ascospores under special conditions of growth. In such a case the fungus should be classified as Ascomycetes when it would show formation of ascus. These fungi are therefore classified on the basis of asexual spores or conidia which are relatively specific for a particular group of organisms. The spores may be hyaline or coloured, unicellular or multicellular. The wall of the spore may be smooth or rough, thin or thick. According to the system of Saccardo (1884) Fungi Imperfecti are divided into three orders as follows:

Conidiophores form acervuli in the substrate **MELANCHONIALES**

Conidiophores form cup-shaped receptacles called pycnidia

SPIHAEROPSIDALES (Homales)

Conidiophores free and do not form fruit bodies **HYPHOMYCETALES**

(Moniliales)

Fungi producing diseases belong to the last group i.e. Hyphomycetales and a medical mycologist is specially interested in this particular group of fungi. Moniliales or Hyphomycetales have been further divided into the following families:

(1) Moniliaceae or Mucdinaceae—conidiophores and conidia are hyaline and bright in colour

(2) Dmatraceae—conidiophores and conidia are dark but conidia may sometimes be hyaline on dark conidiophores

(3) Stilbiaceae—conidiophores are fasciculate, parallel and form corremia

(4) Tuberculariaceae—Conidiophores form a globous sessile body called sporodochium. Another may be added to the above

(5) Mycelia sterilia—the members of this group do not produce any characteristic spore

Fungi imperfecti that are of medical interest belong to two families viz. Moniliaceae and Dmatraceae (dark spores) of the order Moniliales. Further classification of Saccardo is based on the character of spores whether the spores are one celled or two celled or whether the spores show cross septa or both longitudinal or cross septa (muriform). For a medical mycologist it is convenient to classify Fungi Imperfecti according to the classification of Vuillemin instead of one followed by Saccardo. Vuillemin (1910) divided Fungi Imperfecti into three orders on the basis of character of the spores and the fourth order Microsiphonales was later included (1912). According to a later classification by him (1925) the order Microsiphonales (Actinomyces) was included as a family. But according to the classification of Bergey (1948) Microsiphonales have been included in the order Actinomyces under Schizomycetes. Vuillemin divided asexual spores into two main divisions namely thaliospores and conidia. Conidia may be conidia vera and

aleuriospores. Conidia vera are subdivided according to whether they are borne on sterigma or on well defined conidiophores. The conidiophores have further been differentiated into phialides. The spores borne on phialides have been called phialospores. He also recognised Hemisporae which structurally occupy a position between arthrospores and true conidia. Aleuriospores were differentiated from true conidia by the fact that unlike true conidia they are not set free when mature but are liberated only after disintegration of mycelium. These are also called microconidia and differentiated from septate macroconidia which are larger and pluriseptate. American workers are in favour of calling aleuriospores as conidia but it is better to call them aleuriospores (microconidia) and differentiate them from conidia (conidia vera) according to the original classification of Vuillemin so that one could be distinguished from the other. Spores arising from thaliospores have already been described. The classification of Fungi Imperfecti on the basis of Vuillemin's classification (1925) is as follows:

CLASSIFICATION OF FUNGI IMPERFECTI

(After Vuillemin modified)

Order Thaliosporales reproducing by thaliospores

Suborder *Blastosporineae* reproducing by blastospores. This includes nonsporing yeasts like *Cryptococcus* (toruli). Budding mycelia of the form of pseudomycelia are seen in the genus *Candida*.

Suborder *Arthrosporineae* reproducing by arthrospores. e.g. *Geotrichum canidum* *Trichosporium* *Madurella* *Indiella*.

Family *Mycodermaceae*—with usual septate mycelium showing elongated budding forms.

Order Hemisporales reproducing by hemisporae. Genus *Hemispora* // *stellata*.

Order Conidiosporales reproducing by conidia

Suborder *Aleuriosporinae* reproducing by aleuriospores. Ringworm fungi according to Ota and Lingeron (1923) fall in this group.

Suborder *Sporotrichinae* reproducing by conidia but conidiophores are absent. e.g. *Sporotrichum* & *Schenckia*.

Suborder *Sporophorinae* reproducing by true conidia borne on sporophore. Most of the common moulds of the class Fungi Imperfecti belong to this group.

Suborder *Phialineae* reproducing by true conidia borne on phialides (flask shaped sterigma) e.g. *Aspergillus* *Penicillium* etc. But some of these have been found to reproduce by ascospores and in that case they should be classified under the class Ascomycete.

NOMENCLATURE

Under the plant kingdom there are phyla. Each phylum is subdivided into a descending order into classes, orders, suborders, families, tribes, genera and species. Species is further subdivided into varieties when one differs slightly from another. It is very important to note that a lower end member suborder may be a family in the subfamily and a tribe in the subtribe.

The name consists of two parts: the generic part which is a Latin substantive and always written with a capital letter; the specific name of the fungus which is usually a hyphen and is not written with a capital letter. *Penicillium purpogenum*. In this *Penicillium* is the generic name and *purpogenum* is a room-like appearance but the specific name *purpogenum* indicates a pigment which is purple in colour. Let us take another example—*Aspergillus*. The word *Aspergillus* is the generic name. It is so named in honour of the Milanese apostle who has a halo of mistle for baptizing holy water (Latin *Aspergere* = sprinkle). *flavus* indicates the yellow colour of the organism. The species is also named after the name of the discoverer e.g. *Trichophyton schoenleinii*. In those cases in which the author's name is written with an initial capital letter. When citing the name of a species the name of the author who described it originally is often appended to the name of the species. But the name of the author is not written in italics. When the name is mentioned in the text, the name is introduced the name of the original discoverer is put within brackets e.g. *Trichophyton* (Sacc.) Sacc. It is amended from the name *Microsporum* (Sacc.) Sacc. 1833 and expressed as *Trichophyton* (Sacc.) Sacc. (C. Robin) Blanch. 1916. Those who are especially interested in nomenclature refer to the International Rules of Botanical Nomenclature.

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CHAPTER II

MYCOSES

Terminology Diseases caused by fungi are called mycoses (sing mycosis). The terminology used for classification is based on the site affected or the fungus which produces the disease.

(1) Anatomical terminology is used according to the site involved by the fungus viz dermatomycosis onychomycosis otomycosis pulmonary mycosis etc. This terminology is convenient to use when a group of fungi is concerned to produce the disease.

(2) A mycological terminology which is also useful is based on the name of the fungus that causes the lesion e.g. actinomycosis moniliasis sporotrichosis trichophytosis epidermophytosis etc. In certain cases however it is convenient to use a combination of the above two viz broncho moniliasis pulmonary aspergillosis etc.

Classification Human mycoses may be divided into three groups according to the tissue involved.

(a) **SUPERFICIAL MYCOSES** These are caused by epiphytes or ectoparasites which may affect the deeper layers of the epidermis and set up inflammatory reactions. Ringworm which affects the surface of the skin or its appendages like hair or nail is a typical example of such a mycosis. In other instances epiphytes grow on the keratinised surface of the skin and often produce patchy macular lesions with discolorations of the skin without any tissue reaction e.g. pityriasis versicolor. A fungus may produce slight excoriation of the keratinised layer of the skin often due to sweating e.g. erythrasma. Other instances of superficial mycoses are piedra trichomycosis and otomycosis.

(b) **SUBCUTANEOUS MYCOSES** In these granulomas are limited to the subcutaneous tissues and produced as a result of tissue reaction to fungi. The lesions rarely spread via lymphatics or blood e.g. actinomycosis mycetoma chromoblastomycosis sporotrichosis rhinosporidiosis.

(c) **SYSTEMIC MYCOSES** In these the causative fungi multiply in various internal organs and produce infectious granulomas. Diseases of this group are North and South American blastomycosis coccidiomycosis cryptococcosis histoplasmosis moniliasis etc. Rarely sporotrichosis may involve the internal organs and some are inclined to include it under systemic mycoses as the lesions spread through lymphatics. Many fungi causing systemic mycoses may produce primary lesions in the skin and secondary lesions in the internal organs and vice versa.

The classification of mycoses is not yet satisfactory as some would like to accept an arbitrary clinical classification as mentioned above in absence of a natural systemic classification. The main difficulty in the clinical classification is the plurality of the parasite for a particular clinical condition and polymorphism.

of the lesion produced by a fungus in different tissues. It is difficult indeed at this stage to give a clear cut list of mycoses in relation to the tissue involved. A natural classification on the basis of systemic position of fungi would have been an ideal one but the difficulty in that is that the systemic position of some of the pathogenic fungi like *Rhinosporidium seeberi* or *Coccidioides immitis* have not yet been determined. Again fungi of the same systemic group may produce lesions widely variable from one another. In this book diseases are arranged as far as possible on the basis of natural classification with the full knowledge of its drawbacks and difficulties.

Epidemiology—The epidemiological factors of internal mycoses are still unexplored. Recently light has been thrown on the subject of systemic mycoses especially in relation to their epidemiological aspects. In this connection it should be mentioned that systemic mycoses are specially limited to certain areas of the world with their peculiar materiological and other environmental conditions. Gilchrist's blastomycosis is peculiar to North America but only a few cases have been reported from Canada and England although stray unauthenticated cases have been reported from India and other parts of the world. Paracoccidioidosis is particularly limited to South America. Coccidiomycosis is a disease of southern parts of United States.

Dimorphism—Dimorphism is a feature peculiar to fungi of many systemic mycoses. In many of these the fungus shows a morphological adaptation to a simple unicellular yeast form suitable for rapid reproduction and invasion of the human tissue but in artificial cultures in Sabouraud's glucose agar at room temperature they show mycelial forms with formation of conidia. When the cultures are grown on blood agar at 37°C yeast forms appear as in the human body.

The dimorphic characters of the fungi are shown below —

Organism	Disease	Yeast form in blood agar at 37°C	Mycelia in Sabouraud's glucose agar at room temperature
<i>Blastomyces dermatitidis</i>	Blastomycosis	+	+
<i>B. brasiliensis</i>	Paracoccidioidal granuloma	+	+
<i>Sporotrichum schenckii</i>	Sporotrichosis	+	+
<i>Histoplasma capsulatum</i>	Histoplasmosis	+	+
<i>Coccidioides immitis</i>	Coccidiomycosis	Not seen	+

+ = Present

Sources of Infection Fungus infection often occurs from extraneous sources particularly from the environment e.g. wood soil etc. Incidence of Madura foot has often been found as a result of trauma caused by splinters of wood or as a result of thorn prick.

Sporotrichosis is often seen in gardeners and wood-cutters. The parasite *Sporotrichum schenckii* has been found as a saprophyte in various vegetable substrates nests of insects hide of horses which suffered from naturally acquired sporotrichosis. Du Toit (1942) observed in South Africa that the fungus has a saprophytic existence in timbers in mines and infection was contracted by mine workers from infected wood.

Chromoblastomycosis is caused by *Phialophora verrucosa* *Hormodendrum p. brasili* and *H. compactum*. Conant (1937) observed that *P. verrucosa* (*Cadophora americana*) occurs as saprophytes in decaying wood. *H. pedrosoi* has close resemblance with saprophytic species of *Cladosporium* present in the soil. It therefore leaves little doubt that this infection occurs as a result of contamination of the skin by soil or decaying vegetation after a trauma.

Blastomycosis *dermatitidis* has been observed to grow saprophytically in the timber of North America and the pulmonary infection occurs in the majority of the cases by inhalation of spores from the saprophytic sources but in other cases inoculation of the fungus into the skin produces skin lesions.

Cryptococcus neoformans the causative organism of cryptococcosis has been found in milk products and other food stuffs. The infection in these cases has been suggested to occur through the oral mucous membrane or the skin. According to Benham exogenous *Cryptococcus* differs from the endogenous one which is responsible for producing cryptococcosis.

Reservoir *Coccidiomyces* is highly infectious and common in its mild primary stage in the desert areas of South Western States of North America. It has been shown by the recent work that rodents harbour the infection. They act as animal reservoir of the disease and the desert soil is contaminated by the reservoir hosts. The spores of the fungus when carried by the wind are inhaled by man causing primary coccidiomycosis of the lung. The infection is rarely acquired through the skin.

Histoplasma capsulatum often produces systemic mycosis. It also affects the skin producing skin lesions. It is a naturally acquired disease in man and dogs but there is no definite evidence as yet to say that the dog is the reservoir host.

Host Specificity Ringworm of the hair namely microsporosis and endothrix trichophytosis are specially limited to children and the disease is spontaneously cured at the attainment of puberty although there are instances in which endothrix trichophyton was seen to persist in some cases after the age of puberty. Infection with *Microsporum audouinii* has not yet been seen in an Indian child although it is quite a common incidence in Anglo Indian and European children in India. *Trichophyton violaceum* infection which is common in India is seen in Mohammedan children and no case has been seen as yet amongst Hindus.

Microsporosis and some cases of endothrix trichophytosis are self-terminating and the disease often disappears at the advent of puberty. This has been explained by some authors due to changes of the hair brought about by the endo-rine secretion at puberty and this makes the hair resistant to those infections. Epidermophytosis is a common disease at the advent of puberty but it is rare in childhood. *Microsporum canis* causes infection of cats and dogs and human subjects (both children and adults) get the infection which produces intense inflammatory reaction causing a condition of kerion.

Endothrix trichophyta viz *T. violaceum*, *T. tonsurans* etc are pathogenic to children and the infection spreads from one child to another. *T. discoides* is a pathogen of cattle whereas *T. mentagrophytes* (granular) is a pathogen of horses and animals. It may also infect man causing tinea barbae or tinea capitis. A variety of the same fungus is seen well adapted in cases of interdigital ringworm viz *T. interdigitale* but they often produce inflammatory lesions like blisters on the insteps of the foot. On the other hand the same fungus may show a fairly moderate degree of adaptation to human hosts. In such a case the infection may persist in the skin without any inflammatory change but with recrudescence of the disease under favourable conditions of moisture and temperature.

Relation of host to parasite. The fungi that are well adapted to man viz *M. audouinii* may cause spread of ringworm in an epidemic form in boarding schools but *M. canis* an animal parasite is ill adapted to human hosts and reaction of the host is very severe as shown by a process of inflammatory reaction producing a condition of kerion.

Histopathology. This is studied in systemic mycosis where it essentially forms an infectious granuloma. In the immediate vicinity of the parasite there is death of the tissue with abscess formation. Many of these abscesses become surrounded by polymorphonuclear neutrophils and granulation tissue consisting of mononuclear cells with giant cell formation and a dense layer of fibrous tissue. The reaction however varies according to whether the lesion is a primary or secondary one. In primary blastomycosis of the skin the lesion is essentially a granuloma with giant cell formation whereas a metastatic lesion often shows abscess formation. However in experimental infection in the laboratory the type of lesion widely varies with the dosage mode of inoculation etc.

Antibodies. In bacterial infection circulating antibodies like agglutinating precipitin and complement fixing antibodies form a marked feature but in mycoses cellular reaction is characteristic and the antibodies which are often cell-fixed are responsible for sensitisation of the individual and produce allergic reactions. The cell wall of the fungus is thick and its intracellular protein hardly diffuses into the tissues. The reactions are often due to liberation of substances owing to autolysis of the fungus. The serological tests that might be positive in fungus infections are agglutination, precipitation and complement fixation.

Agglutination.—Agglutination reaction has been performed in sporotrichosis

schenckii from another : Sera of sporotrichosis patients agglutinate spores of *S. schenckii* in a high titre of 1 in 300 to 1 400. But it has the disadvantage in that the sera of thrush cases agglutinate spores of *S. schenckii* even in a higher titre than they would do with the suspension of *Candida albicans*. Sera of patients suffering from actinomycosis also agglutinate spores of *S. schenckii*. Agglutination reactions are really unsatisfactory for most of the mycoses.

Precipitation—Precipitin has been detected in severe cases of coccidiomycosis but it is negative in mild cases. This test is of little use in mycoses.

Complement fixation The test was found to be positive by Widal et al (1910) in sporotrichosis but DuToit (1942) found this test to be unreliable. Saline suspension of the yeast form of *B. dermatitidis* as an antigen has been claimed to have given a positive complement fixation test with diluted sera in cases of North American blastomycosis and there is no cross reaction with other fungi. But it is negative in mild and cutaneous cases even with undiluted sera. Complement fixation test also becomes positive in cases with extensive lesions of Coccidio mycosis as in the cases of North American blastomycosis but the test has not been confirmed in cases of South American blastomycosis.

Allergy and skin hypersensitivity Fungi have little invasive power as they often attack traumatised or keratinised tissues of the body. To be invasive therefore the fungi should either increase in their virulence or there should be some environmental tissue changes. Increase of virulence could not however be demonstrated in experimental inoculation in animals and therefore it is more likely that there is alteration in the tissue of the host.

Local changes in infections are due to liberation of endotoxin which often causes destruction of the tissue resulting in extension of the lesion. But more important changes produced by fungus infection are the altered reactivity of the tissue leading to allergy and skin hypersensitivity. In this latter condition the fungus due to its continued existence at the site of primary infection sensitises the cells of the body and thus the host becomes susceptible to an accelerated tissue reaction. In this state if the fungus or its product is introduced into the host an allergic reaction is liable to occur with rapid spread of the disease. In dermatophytosis the hypersensitiveness is noticed when the spores or mycelia enter the circulation from the site of primary infection. This is characterised by the appearance of skin eruptions which are also known as id reaction. Sensitisation is detected by various intradermal injections of extracts of fungi. These cutaneous reactions may become positive in cases with previous infection with the same fungus or an antigenically allied fungus. So long as the cutaneous reaction is positive it indicates presence of sensitisation. But the antigen (allergen) in fungi is often of the nature of a group antigen rather than a specific one and this is the reason why an antigen which is common in more than one fungus may produce a cross reaction. Therefore a negative reaction is more important than a positive one. The following skin reactions have been utilised in various fungus diseases.

Trichophytin In experimental works in guineapigs it has been repeatedly demonstrated that sensitisation occurs only when a living culture of ringworm fungus is injected into the skin. Injection of dead culture or the fungus extracts i.e. trichophytin does not provoke dermal sensitivity, nor injection of living culture in tissues other than skin produces this sensitivity. The degree of sensitivity depends upon the species that infects the skin. It is very much marked with animal species which cause deep seated lesions namely *T. mentagrophytes*, *T. quinckeraum* and *M. canis*. The reaction is minimum with human species like *M. audouinii*, *T. tonsurans* and *T. schoenleini*. By the skin test and by sensitisation and quantitative desensitisation of the guineapig's uterus by Schultz Dale technique Jadassohn et al (1937) demonstrated that trichophytin contained a group antigen and some minor specific factors. Therefore trichophytin test can not be considered as a specific but a group test. This should not therefore form the sole basis of diagnosis.

Various preparations of trichophytin are available and any of the standard preparations may be used for the test - namely Metz 1:100 Lederle 1:30 or dermatrichophytin Muller and Phipps (India) Ltd. It is difficult to standardise a home made trichophytin. The reaction is judged by erythema and induration produced by the intradermal injection of trichophytin.

VALUE OF THE TEST Trichophytin has been used diagnostically specially in suspected cases of id reactions and also therapeutically in deep seated ringworm infections. The skin reaction becomes positive in the majority of deep seated ringworm infections. After a considerable scrutiny of the results by various authors it has been concluded that negative reaction is of greater value to exclude an inflammatory lesion of dermatophytic origin than a positive trichophytin test. If the lesion is of long duration the fungus cannot be detected microscopically and culturally and the trichophytin test is negative the inflammatory lesion may be declared not to be of dermatophytic origin.

Coccidioidin Dr C. T. Smith prepared coccidioidin from strains of *Coccidioides immitis* in a synthetic liquid medium. In systemic involvement of coccidiomycosis marked skin hypersensitiveness develops. The reaction is highly specific but a small number of persons who had no history of disease or contact or no history of residing in an endemic area were found to react to coccidioidin. This has not been explained as yet. Therefore the negative test is valuable in the diagnosis as it excludes the infection in cases other than those who are in the preallergic stage of the disease or in a state of anergy resulting from overwhelming infection. In cases of systemic mycosis the test is useful epidemiologically to map out endemic areas.

Blastomycin (Extracts of *B. dermatitidis*) The test is specific. According to Martin and others every patient suffering from active lesions of blastomycosis may not react to the skin test performed by the intradermal injection of blastomycin. Mild cases with skin lesion often give negative results. Normal people usually do not show the positive skin reaction. Hypersensitiveness to it, as

determined by the skin test is a contraindication for iodide therapy. Cases of blastomycosis should be desensitised before they are treated with x ray or iodide.

Histoplasmin It is filtrate of synthetic broth culture of the fungus *H. capsulatum*. Histoplasmin has been used in epidemiological studies and found to be useful for detecting benign type of self limiting pulmonary histoplasmosis in endemic areas. Histoplasmin skin test becomes positive in the endemic areas of histoplasmosis in cases of non tuberculous pulmonary calcification. Cross sensitisation reactions may be noted for blastomycin and histoplasmin. Patients of coccidiomycosis often show a positive skin reaction to histoplasmin.

Sporotrichin Intracutaneous test with an extract of *S. schenckii* is useful when the test is positive. A negative result rules out the diagnosis of sporotrichosis. Occasionally only there may be a false positive reaction. According to Du Toit a positive reaction always occurs in cases of sporotrichosis. The appearance of sensitivity has been found to be on the fifth day after inoculation to a volunteer.

Oidiomycin This is a commercial vaccine prepared from *Candida albicans*. According to Lewis and Hopper skin sensitivity test with oidiomycin is of little value. Sensitisation of the skin is possible from a focus in the intestinal tract or from any other lesion which had subsequently resolved. He mentioned that of the 42 cases of cutaneous moniliasis a positive response was obtained in 57 per cent of cases. Again out of 91 patients with an infection due to a fungus other than *C. albicans* 45 per cent showed a positive reaction whereas out of 192 patients without any evidence of fungus infection 46 per cent reacted to oidiomycin. It can therefore be concluded that the test has little practical value in the diagnosis of moniliasis.

Asthma Various spores of common moulds have been incriminated as etiological agents in asthma. These spores when inhaled with dust sensitise the subject and the sensitisation can be detected by cutireactions. Spores that might cause asthma are those of *Puccinia graminis* (wheat rust fungus) various species of *Aspergillus*, *Alternaria* and others. It has also been suggested that similar fungi might produce allergic eczema in those cases of asthma.

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CHAPTER III
FUNGI PATHOGENIC TO MAN
TABLE II

Class	Order	Suborder	Genus	Species
Schizomycetes (Fission fungi)	Actinomycetales		Actinomyces Nocardia	<i>A. boe</i> <i>N. m. d.</i> <i>N. k. tol. t.</i> etc
Fungi Imperfecti	Thalloporales	Blasto-porineae	Cryptococcus Pityrosporum Blastomyces Coccidioides Histoplasma Phaeophora Candida Malassezia Gyrodium Trichosporum Malassezia Trichia	<i>C. n. f. m. t.</i> <i>P. o. r. t.</i> <i>B. d. m. t. d.</i> <i>B. b. r. l. t.</i> etc <i>C. m. m. t.</i> <i>H. c. p. s. lat. m.</i> <i>P. u.</i> etc <i>C. l. b.</i> <i>M. f. f.</i> <i>G. d. d. m.</i> <i>T. b. g. l.</i> <i>M. m. y. c. t. m.</i> etc <i>T. m. n.</i> <i>T. e.</i> etc
	Hemiporales		Hemiphysa	<i>H. t. l. l. a.</i>
	Coniophorales	Microsporiae	Microsporum Epidermophyton Trichophyton	<i>M. d.</i> etc <i>E. f. m.</i> <i>T. t.</i> <i>T. s. h. l.</i> etc
			Glomerula	<i>G. g. p. h.</i> <i>G. a. m. n.</i> etc
			Trichium	<i>T. t. l.</i>
		Sporotrichineae	Sporotrichum	<i>S. h. k.</i>
Ascomycetes	Ascomycetales		Aspergillus Penicillium Streptomyces Alliaria Lecanora	<i>A. b. f. f. d.</i> <i>A. f. m. t.</i> <i>A. n. e.</i> etc <i>P. m. y. t. m.</i> <i>S. n. d. l.</i> <i>A. b. y. d.</i> <i>P. h. t.</i>
Phycomycetes	Mucorales		Mucor Rhizopus	<i>M. s. m. b. f.</i> <i>R. y. R. n. g.</i>
The exact taxonomic position is not determined			Phaeosporium	<i>P. b. t.</i>

Some species are not included and Phyllaea (Fungi Imperfecti)

STUDY OF PATHOGENIC FUNGI

Pathogenic fungi are studied under the following heads —

- 1 Direct microscopic examination
- 2 Culture for isolation and study of giant colonies
- 3 Microculture and hyphal fusion
- 4 Biochemical reactions
- 5 Filtered ultraviolet light (Wood's light)
- 6 Histopathology
- 7 Animal inoculation
- 8 Cutaneous tests (See Chapter II)
- 9 Serological reactions (See Chapter II)

Direct microscopic examination Direct microscopic examination of the suspected material is one of the most important methods available for investigation of fungi diseases. Examinations of the hair scraping from the glabrous skin or nails show presence of the fungus in the ringworm lesion. Any keratolytic solution which dissolves and clarifies hairs, scales and nails may be utilised for this purpose. Usually 10 or 40 per cent aqueous solution of potassium hydroxide is used as a keratolytic agent.

Sodium sulphide is used by the author as a routine keratolytic agent*. In a coverslip preparation with this solution scales or hairs when heated for a minute are quickly keratinolysed in about 5 minutes but for nails it takes about 3 hours to dissolve it completely. This solution has the advantage over that of caustic potash in that (i) it clarifies the cells without destroying the fungus (ii) it shows the fungus clearly and ringworm fungus may be spotted out under the low power and then confirmed by the high power.

As an alternative method the fungus can be detected after staining with McGuire's stain*. By this method the fungus are stained purple. It is very easy to stain fungi by this method when they are present in the thin scales of *tinea imbricata*, *tinea versicolor* and *seborrhoea capitis*.

Another method is to treat scales and scraping of hair in 5 to 10 per cent solution of caustic potash in a watch glass. The material is then washed in water for a few minutes, mounted on a slide under a cover slip in a drop of heated lactophenol with cotton blue. Hairs can also be mounted permanently.

Fur or other exudates Fur from an itchy exudate from an ulcer or spottum may be examined in the above way but they may be examined satisfactorily by a coverslip preparation with McGuire's stain. This stains the fungus purple. Granules in actinomycosis and nocardiosis are easily examined by crushing a granule in a drop of stain under the cover slip. It stains the fungus beautifully and the leucocytes as well.

Cerebrospinal fluid The cerebrospinal fluid is centrifuged and examined in any of the above methods or by fixing and staining smears with polychrome methylene blue or Leishman's stain. Capsules of *Cryptococcus neoformans* can be well demonstrated by Indian ink in a coverslip preparation.

Culture Ringworm fungi may be detected under the microscope but for the identification of species the study of macroscopic and microscopic characters of the growth is necessary. Sabouraud's proof medium is used for primary culture and subcultures. As it is difficult to obtain the ingredients of the original formula different countries have modified the formula for this medium which gives a growth similar to one obtained in Sabouraud's proof medium.*

For isolation of fungus from grossly contaminated material (ringworm of the nail) it is better to use Sabouraud's proof medium containing 1:400,000 of gentian violet*. The method is very satisfactory and secondary contamination is practically absent when this method is adopted. Giant colonies are studied in Sabouraud's proof medium in Erlenmeyer flasks*.

For isolation of fungi from cases of blastomycosis, histoplasmosis and coccidiomycosis inoculate blood agar and glucose agar tubes and incubate them at 37°C but inoculate another set on Sabouraud's glucose agar and incubate them at room temperature. For other media see appendix.



Fig 1a—Well slide



Fig 1b—Hollow ground slide



Fig 1c—Van Tiegh method



Fig 1d—Microculture in well slide

Microculture For this hanging drop cultures are studied in well slides containing square slabs of stone about 2.5 cm square and 5 mm in thickness with a central circular hole of 1.5 cm in diameter. The slab is cemented on a microscopic slide (Fig 1a). The slide and the coverslip should be sterile. The coverslip should be grease free. Both can be wrapped in craft paper and autoclaved. Sabouraud's semisolid agar (0.5 per cent agar) or any other suitable medium may be used for the study of the morphology of the fungus. The agar is melted in a boiling water bath and a drop of the medium is spread in the centre of the grease free coverslip with the help of a platinum loop. As soon as the medium is cool the agar sets on the coverslip and a tiny bit of inoculum of the fungus to be studied is placed on agar. The coverslip is turned upside down so that the inoculum is in the centre of the well hanging down from the coverslip (Fig 1d). The edge of the coverslip is quickly sealed with a mixture of hard and

soft paraffin in a proportion of 1:2. The mixture is melted and spread at the edge of the coverslip with the help of a camel hair brush. The fungus grows in the medium and is studied for about 6 weeks. Instead of media thick wheat or starch solution scales hair shavings from horn or hoops may be inoculated with the fungus with a drop of sterile water in the chamber. Instead of well slides a hollow ground slide (Fig. 1b) or a Van Tieghem cell (Fig. 1c) can be used for the same purpose. The progress of the growth is studied from time to time for reproductive organs.

Culture mounts are prepared from the hanging drop culture or by taking a bit of growth from agar. The agar is teased separated from the growth and gently washed away. The growth is then mounted in lactophenol—and sealed. Another useful method is to spread a drop of melted agar under a coverslip. The inocula from a culture are introduced on four sides of the coverslip. The preparation is preserved in an improvised sterile, moist chamber. From time to time the reproductive organs are studied. When these reproductive organs are fully developed the coverslip is taken out and a culture mount is prepared.

As an alternative method materials of dermatophytes could be inoculated on agar drops. Dr. Curry of Manchester University demonstrated a simple method for this purpose. A sterile Petri dish is taken and on the inverted lid drops from a melted Sabouraud's agar tube dipped in a boiling water bath are dropped rapidly from a Pasteur pipette. Drops are put at a distance of about one inch or so in rows and columns. Agar drops solidify in no time. These drops are then inoculated with the infective material after a preliminary treatment in alcohol for about five minutes. About 20 inocula may be put in one plate. Put a thin layer of sterile distilled water at the bottom of the Petri dish and put back the inoculated plate in position. Thus the agar drops do not dry until water is exhausted by evaporation. Water may be replenished if necessary from time to time. The lid of the Petri dish can be everted placed on the stage of the microscope and examined for the reproductive organs.

Hyphal fusion. This was originally devised by Davidson et al (1932). In this two inocula of the fungi to be studied are placed side by side in a hanging drop culture in well slide without touching each other. The fungus grows in the medium. When they are allowed to grow mycelia of similar strains of same species only fuse with each other but mycelia of different strains or species do not show any hyphal fusion.

Biochemical reactions. Biochemical reactions are studied after isolation of organisms like *Candida albicans* and other yeast like fungi. Bacteriological sugar tubes are used for this purpose. Fermentation results are fairly constant for these organisms and thus may help in the determination of species. Proteolytic activity of fungus is characterised by liquefaction of organulated serum gelatin or peptonization of milk.

Filtered ultraviolet light. This has been extensively used by American workers. By this method are studied in lesions and in culture by using

ultraviolet light passing through Wood's filter. This filter allows passage of ultraviolet light of wave length of about 3650 Å. Certain fungi or infected materials show fluorescence in this light. The method has been utilised for the detection of infection caused by *Microsporum audouinii*, *M. gypseum* and *M. canis* in the routine diagnosis of ringworm of the scalp. The changes detected under Wood's light are mentioned in respective chapters.

Histopathology. Fungi that invade deeper tissues or internal organs show formation of granulomas often with giant cell formation. The characteristic lesions are dealt with in respective diseases.

Animal Inoculation. Inoculation of mice with infected material or culture is very important specially in cases of blastomycosis, coccidiomycosis, cryptococcosis and sporotrichosis. This method should therefore be resorted to along with usual examinations, namely direct microscopic examination and culture. The method is also useful to isolate the organism in pure form from contaminated materials. For inoculation susceptible animals like mouse, rat, rabbit or monkey may be used. Again when an organism is isolated, animal inoculation is useful for finding out the pathogenicity. Some of the saprophytes or contaminants can thus be excluded by adopting the method of animal inoculation.

In ringworm infection when the fungus is isolated in culture, animal inoculations are undertaken as a routine measure for pathogenicity test. This is done locally by shaving the skin on the back or abdomen and the suspension of the culture is applied on the part after scarification. Fungi of animal sources usually produce lesions, whereas the lesions produced by human species are spontaneously cured in about 10 days.

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ORDER ACTINOMYCETALES

The organisms of the order Actinomycetales occupy a position intermediate between bacteria and fungi. But according to the present conception they are considered as Schizomycetes (Bergey 1948). They are 1 μ or less in diameter and include *Mycobacterium*, *Actinomyces* and *Nocardia* but unlike bacteria the organisms of the last two groups show branching. On the other hand in some actinomycetes mycelial filaments divide into fragments resembling bacillary (diphtheroid) and coccoid forms.

CLASSIFICATION (Waksmun and Henrici 1943)

- A Mycelium rudimentary or absent Family *Mycobacteriaceae* Chester
1 Organisms acid fast Genus *Mycobacterium* Lehmann and Neumann
- B True mycelial forms showing branching
- 1 Vegetative mycelium divides into fragments (bacillary or coccoid forms)
 Family *Actinomycetaceae* Buchanan
- (a) Anaerobic or microaerophilic parasitic nonacid fast
 Genus *Actinomyces* Harz
- (b) Aerobic, partially acid fast or nonacid fast
 Genus *Nocardia* Trevisan
- II Vegetative mycelium does not divide into bacillary or coccoid fragments
 Family *Streptomycetaceae* Waksman and Henrici
- (a) Aerial hyphae form conidia in chains
 Genus *Streptomyces* Waksman and Henrici
- (b) Aerial hyphae show terminal spores on short sporophores
 Genus *Micromonospora* Orskov

TABLE III TABLE SHOWING PATHOLOGIC EFFECTS OF ACTINOMYCETES AND NOCARDIA

Organism	Disease	Distribution
AFROBIC <i>Actinomyces bovis</i>	Actinomycosis in man and cattle	Cosmopolitan chiefly in U S A
AFROBIC <i>Nocardia madurae</i> and allied organisms	Mycetoma	India, Egypt and a few from U S A
<i>A. asteroides</i> (<i>A. dispersoides</i>)	Pseudotuberculosis and deep-seated abscesses	Cosmopolitan
<i>A. keratolytica</i>	Superficial kerion-like lesions of the skin	India
<i>A. minutissima</i>	Erythrasma	Cosmopolitan specially in the tropics
<i>A. tenuis</i>	Nodules of the hair	Cosmopolitan specially in the tropics

for m m k u s t p r i f r e a t t e p f e r d h o m e n i l g m n i t e m m
con i c e d t b e a n c o p t i v e

ACTINOMYCES BOVIS Harz 1877

Synonyms *Actinomyces israeli* Dodge 1935 *Discomyces bovis* Rivolta 1878 *Nocardia actinomyces* Trevisan 1899

History Ray fungus was first demonstrated by Von Langenbeck (1845) in a case of Pott's disease in a young man. Similar organism was reported by Lebert (1846) and Davaine (1859). Sulphur granules were described by Rivolta (1868) in actinomycotic lesions of cattle and the causative organism was called *Discomyces bovis*. The disease in cattle was described by Bollinger (1877) and he called it actinomycosis, Harz in the same year named the organism of lumpy jaw *Actinomyces bovis*. Israel (1878) identified lumpy jaw of cattle with human actinomycosis and Wolff and Israel (1891) cultivated it for the first time and established its pathogenicity.

Habitat *A. bovis* is naturally found in teeth, gum and tonsils of man and in actinomycosis of the jaw, lungs and intestine. In cattle, sheep and pig it causes lumpy jaw (Pl II Fig 1). They are strict parasites and not found in nature apart from the parasitic habitat.

Morphology It is a nonmotile, nonsporing, nonacidfast Gram positive filamentous organism with branching (Pl II Fig 2). The filamentous mycelia are 1 μ or less in diameter. They show bacillary forms after fragmentation. In tissues the organism forms granules which show tangled mass of mycelia and at the periphery of the granules filaments show terminal swellings specially marked in cattle assuming a characteristic club like appearance. The ray like arrangement is due to the clubs. Clubbed mycelia are Gram negative and the tangled mycelia are Gram positive. On staining the tissue section the filaments are stained with haematoxylin and margin of the clubs with eosin.

Cultural characters *A. bovis* grows under anaerobic or microaerophilic condition. In primary culture the growth is poor and slow. It grows in ordinary media but the growth is better in media containing blood, serum or glucose. Brain heart infusion agar (Bacto) is useful for plating. Subculture is easy to grow. No pigment is formed. Rough and smooth forms are recognised, the latter are recovered directly from the oral cavity or from actinomycosis of animals. Optimum pH is 7.2 to 7.6 and the optimum temperature for growth is 37°C.

In glucose agar *Shake culture* the maximum growth is found at a depth of 1 to 2 cm below the surface in a zone where there is only a trace of oxygen and optimal concentration of carbon dioxide, proving that it is microaerophilic (Pl II Fig 3). In *solid media* the colonies are raised, nodular, cream coloured, opaque, heap up about 2mm in diameter and show a rosette form, irregular in outline and firmly adherent to the medium (Pl II Fig 4a). In *liquid media* growth occurs as fuzzy colonies after incubation for several days. Organisms from culture show short bent branching filaments with V or Y forms, often with swollen ends.

Biochemical reactions Several sugars are fermented including glucose and lactose with production of acid only but these reactions are of little diagnostic value. Most of the strains are not proteolytic, do not liquefy gelatin or form

indole These are non haemolytic and do not reduce nitrates Litmus milk is unchanged

Resistance It is killed at 60 C in one hour and susceptible to disinfectants It is difficult to maintain the stock cultures but they may remain alive for several months when kept dried in vacuum Sulphonamides and penicillin are active *in vitro* and are responsible for cure of about 50 per cent of cases

Antigenic structure Strains of *A. boydii* isolated from lesions in man and animals and from the normal mucous membrane show wide range of variation in morphology biochemical reactions etc Serologically they are heterogeneous but their differences do not justify to establish separate new species The pathogenic organisms are classified as one species i.e. *A. boydii*

Animal inoculation It is pathogenic when inoculated into rabbits and guinea pigs producing typical granulomatous lesions containing granules *A. hominis* (Bostroom) which is a saprophyte, grows aerobically and fails to produce any lesion by animal inoculation

ACTINOMYCOSIS

This is a chronic infective granuloma caused by *A. boydii* characterised by formation of nodules which spontaneously ulcerate form sinuses and discharge pus containing typical sulphur granules It primarily affects the jaw bones or intestine but may spread haematogenously to other parts of the body

Signs and symptoms The incubation period is not definitely known but it varies from a few days to a few weeks The lesions are generally painless The disease starts as a firm purplish nodule which gradually enlarges softens to form an abscess The abscess bursts forms a sinus from which sulphur granules are discharged The disease spreads by contiguity and the surrounding tissue becomes studded with nodules The affected area has board like resistance on palpation and shows multiple openings containing granules and sero purulent fluid These multiple openings are due to formation of intractable sinuses The underlying bone may be involved in the process The spread of the disease does not occur through the lymphatics and this is how it differs from other granulomas Metastasis may take place in the liver brain kidney or lungs by haematogenous route The breast or ribs may be involved as a process of extension from the lungs

It is customary to classify actinomycosis into cervicofacial thoracic abdominal and skin types according to the site of involvement

Cervicofacial actinomycosis is the commonest type (Pl II Fig 5) It accounts for 80 per cent cases The route of infection is the mucous membrane of the mouth and frequently it starts from a carious tooth or tonsil From the site of primary lesion it spreads to the deeper tissues and ultimately to the skin of the face and neck or to the tongue and involve the neighbouring structures Maxillary bone vertebrae or orbit may be secondarily involved

Plate II



1



2



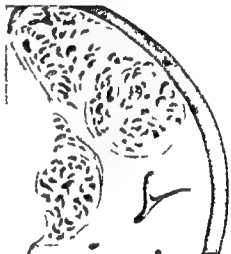
7



5



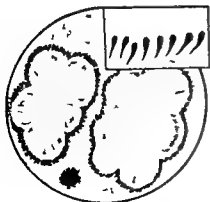
3



6



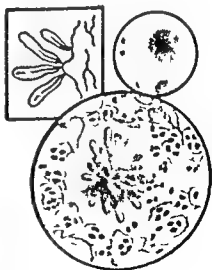
4a



8



4



9

Thoracic actinomycosis accounts for 15 per cent of cases. It originates from the mouth or throat by aspiration of the infective material or as an extension from the abdomen or as metastasis. The lesion is mainly found in the lung where small abscess cavities are formed. Extensive lesions may be seen involving bronchi and their rupture may cause dissemination through the bronchogenous route. From the lung the lesion may spread to pleura, pericardium, heart or chest wall by a process of direct extension.

Abdominal actinomycosis accounts for 20 per cent of cases. It is commonly seen in the caecum and appendix. The abdominal infection is probably derived through the intestinal mucous membrane which may not be involved. Suppurating foci may be adherent to the abdominal wall by a process of fibrosis involving the skin. The liver is commonly involved by a process of metastasis or extension (Pl II Fig 6) and extension of the lesion to the genital tract and kidney is relatively frequent (Pl II Fig 7).

Skin actinomycosis accounts for 15 per cent of cases. It is difficult to say whether skin is primarily affected in every case as actinomycosis of the skin secondary to lesions of the underlying tissue or organ is rather common.

Pathology. In the tissue the organism forms granules which are discharged in the pus. When these granules are crushed and examined with McGuire stain they are seen to be composed of tangled mass of mycelial filaments forming club at the terminal end. These are arranged in radiating manner and hence the name ray fungus (Pl II Fig 8). Clubs are well formed in the lumpy jaw of cattle but not in human actinomycosis. The granules are embedded in pus containing polymorphonuclear neutrophils (Pl II Fig 9). Around the mycelial mass granulation tissue is formed with formation of giant cells and proliferation of fibroblasts. Filled macrophages are present in the peripheral portion of the abscess and this may give yellow colouration of the lesion visible even to the naked eye.

Diagnosis. The clinical diagnosis should be ascertained by macroscopic examination of the granule. Granules are crushed and examined under a coverslip preparation with a drop of McGuire's stain. They show ray fungus or filaments with ray like arrangements. The isolation and identification of the organism can only be made by culture which should be done both aerobically and anaerobically. For this granules are washed repeatedly in saline treated with alcohol for 5 minutes and crushed into small bits. The tiny bits are then inoculated in 1 per cent glucose agar shake culture tubes. Several tubes should be inoculated and incubated at 37°C anaerobically for about a week. Mulberry like colonies about 2 or 3 mm in diameter appear in the depth of the agar. A dense zone of growth is seen about 2 cm below the surface. Bacto brain heart infusion medium with 2 per cent agar may be

Plat II Fig (1) Lumpy jaw in cattle (2) Terminal end of mycelia of *A. I*
(3) Lumpy jaw of cattle (4) Granules of *A. I* on glucose
(5) Lumpy jaw of cattle (6) Actinomycosis of kidney
(7) Actinomycosis of kidney (8) Ray fungus (net—1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 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used for culture under anaerobic condition. Once the anaerobic growth has appeared subcultures may be maintained aerobically in a suitable medium. Serological and cutaneous tests have proved to be unsuccessful.

X-ray diagnosis In the early stage of the disease the skiagram of the jaw does not show any involvement of the bone but later there may be periostitis or true osteomyelitis with destruction of the bone.

Treatment In the early stage when there is no involvement of the deeper tissue excision of the nodule may be possible. In the later stage of the disease all the sinuses should be opened up, scraped and drained. Liq. iod. mitis can be used locally as an antiseptic. Potassium iodide should be administered intravenously or by mouth in massive doses. Initially 10 cc of 10 per cent solution can be administered intravenously and the dose is gradually increased upto the extreme limit of tolerance of the patient. When the lesion is not accessible X-ray therapy has given good results in semi intensive doses with filters. The treatment should be repeated for 3 to 4 weeks. In systemic infection deep therapy is indicated.

Penicillin should be administered intramuscularly in doses of 40 000 units every three hours to a total dose of 6 000 000 units. It should be noted that different strains vary in their sensitivity to penicillin *in vitro*.

Sulphonamides have been administered with good results. Sulphadiazine is administered in doses of 4 to 6 gm per day and the dose is reduced to 2 gm per day after clinical improvement of the patient. The results are however inconsistent with this treatment. X-ray penicillin and sulphonamide treatment may however be combined with advantage.

NOCARDIA

Unlike *Actinomyces*, *Nocardia* are free living aerobic organisms found in soil and its contaminants from soil sources.

History Nocard (1883) first described an organism in cattle called *Nocardia farcinica* by Trevisan (1889). Eppinger (1890) described *N. (Cyllothrix) asteroides* isolated from a case of human pseudotuberculosis with meningitis and brain abscess. Vincent reported *N. (Streptothrix) madurae* (1894) which he cultivated from a case of mycetoma of the foot. Blanchard (1896) put the last two in the genus *Nocardia*. Since these reports many more species were included in the genus *Nocardia*.

Morphology These are Gram positive filamentous organisms 1 μ or less in diameter with branching. Some of them are acidfast (*N. asteroides*) and may be mistaken for tubercle bacilli. In addition to the filamentous forms bacillary and coccoid forms are seen in certain species. In the tissue they form granules yellowish white red or orange in colour with tangled mycelia with or without club formation at the periphery. Bacillary form may be seen in the exudates of body fluids like cerebrospinal fluid, sputum, empyemic fluid.

Culture These are aerobic organisms and grow at room temperature and at 37 C on simple media e.g. glucose agar, Sabouraud's glucose agar, Czapek's

synthetic agar etc. Initially colonies are small and grow slowly and a typical appearance is seen in six weeks. Cultures vary in gross appearance and pigment formation according to the media. They are glabrous wrinkled or cerebriform and the surface is granular or mammillated and closely resemble the growth of acidfast bacilli. The growth may be soft moist and mucilaginous or hard dry and granular. Aerial mycelia which are characteristic of *Streptomyces* are not usually seen. The colour of the growth may be yellowish ochraceous pink red or orange.

Biochemical Biochemical reactions are fairly constant. Some *Nocardia* are proteolytic liquefy gelatin and coagulate milk. They do not ferment carbohydrate or produce indole H_2S or NH_3 . The species are differentiated on the basis of coagulation of milk reduction of nitrates to nitrites power of liquefying gelatin acidfastness and pigment formation.

TABLE IV TABLE SHOWING DIFFERENCE OF COMMON NOCARDIA

Name of species	Granules in lesion	Pigment on Czapek's medium	Growth on Sabouraud's glucose agar	Acid fast	Gelatin liquefaction	Coagulation of milk
<i>N. asteroides</i>	Yellowish white	Yellow to ochraceous orange or red	Wrinkled granular	+	-	-
<i>N. brasiliensis</i>	Yellowish white	Yellow to ochraceous or orange	Often chalky growth cerebriform dry or tenacious earthy odour	+	+	+
<i>M. madurae</i>	Yellowish white	Cream to pink or red	Moist soft wrinkled	-	+	+
<i>N. Pelletieri</i>	Red	Coral red	Glabrous wrinkled moist	-	+	+

NB - nd cate negative and + indicates positive result

Resistance All these are soil organisms and have marked resistance to desiccation. They are readily killed at 60°C for one hour. *N. asteroides* is resistant to streptomycin and penicillin.

Pathogenicity Inoculations of *N. madurae* is not pathogenic to laboratory animals. *N. asteroides* is the only species of the group pathogenic to laboratory animals but strains vary greatly in their power to produce lesions. Death of the guinea pig results after an intraperitoneal injection of a large dose of the organism but a smaller dose injected intravenously causes death of the rabbit producing miliary abscesses. Intramuscular or subcutaneous injection produces local lesion with abscess formation which ruptures spontaneously and becomes cured.

SOME PATHOGENIC SPECIES OF NOCARDIA

NOCARDIA MADURAE (Vincent) Blanchard 1896

Synonyms *Nocardia indica* Kuntze 1893 *Streptothrix madurae* Vincent 1894

The organism is found in the crinules of mycetoma and is commonly seen in Asia and Africa in countries. The grains are yellowish white in colour.

Morphology The crinules are composed of thin mycelial filaments with a few or no club-shaped swellings at the periphery. Some mycelia show chains of small conidia at their extremities. The organism is not acidfast.

Culture *N. madurae* is an aerobic and grows in ordinary media. The growth occurs at room temperature and at 37°C. In liquid media it grows as tiny granular puff balls which rest at the bottom of the media. The supernatant fluid remains clear when there is no contamination. In solid media like Sabouraud's glucose agar raised colonies are seen giving an irregular mammillated surface and a pink colour often develop which gradually becomes red (Pl. III Fig. 1). The size of the colony is about 1.5 cm after six weeks. Old cultures show fragmentation of hyphae which serve as reproductive structures.

Animal inoculation Animal inoculation shows negative result and inoculation to rabbits may cause only a temporary local lesion.

NOCARDIA ASTEROIDES (H. Pinner) Blanchard 1896

N. asteroides produces mycetoma-pulmonary lesions (pseudotuberculosis) in cats of the brain and meningitis. The grains are yellowish white in colour. They are intimately related to *Mycobacterium tuberculosis*. They are very fragile and easily disintegrate when they show bacillary forms. They are acidfast and resemble *Mycobacterium tuberculosis* but unlike *Mycobacterium tuberculosis* they grow faster.

It is an obligatory aerobe and grows quickly on ordinary laboratory media at room temperature and at 37°C. On Sabouraud's glucose agar the colonies are glutinous, folded and the colour varies from pale yellow to deep orange. On medium with examination the colonies are seen to consist of tangled delicate branching hyphae. Bacillary form is also seen. It can be readily differentiated from *Mycobacterium tuberculosis* by culture and simple inoculation.

NOCARDIA BRASILIIENSIS (Lindholm) Chalmers & Chalmers 1913

It is synonymous with *N. m. vicaria* which has been reported to produce mycetoma in Mexico.

N. brasiliensis produces pulmonary lesions which may spread to other parts of the body. The granules are yellowish white in colour. Hyphae are thin delicate and fragile. They are Gram positive and acidfast. Growth on Sabouraud medium are often chalky cerebriform friable tenacious with an earthy colour. The clinical picture is indistinguishable from those of *N. asteroides*.

NOCARDIA PELLUTHERI (Loverin) Pinnus 1912

N. pellutera causes mycetoma with red granules with or without club formations. The hyphae are thin delicate. They show a glutinous wrinkled red red growth. Fragmentation of hyphae is not usually seen. They are Gram positive but not acidfast. They liquefy gelatin in 1% sterile milk.

DISEASES PRODUCED BY NOCARDIA

- 1 Mycetoma
- 2 Superficial nocardiosis of the skin
- 3 Erythrasma
- 4 Trichomycosis nodosa

MYCETOMA

History Mycetoma is commonly known as Madura foot because of its high incidence in the district of Madura in Madras. The condition has been known in India since ancient days and mentioned in Ayurveda as *Padavalnicum* a disease in which the foot is swollen and a kind of fleshy tumour develops like an hill with discharge of peculiar fluid. Colbrook (1844) of Madura clinic described the disease as Madura foot. The fungus nature of the disease was first described by Vandyke Carter (1874) who clearly established it as a distinct clinical entity and named the disease mycetoma (fungus tumour). Vincent (1894) in North Africa succeeded in culturing the fungus from mycetoma of oehroid variety. Pinov (1913) used the term mycetoma to include two etiological groups namely true mycetoma caused by fungi having larger hyphae and actinomycotic mycetoma caused by *Actinomyces* and *Nocardia*. Chalmers and Archibald (1916) accepted this classification and divided Mycetoma into two subgroups Actinomycoses and Maduromycoses (true mycetoma). It is therefore customary to divide mycetoma into these two etiological types and this classification has been generally accepted. Gammel (1917) in a review of mycetoma mentioned thirteen species of *Actinomyces* of the actinomycotic group whereas in the Maduromycotic group he included 19 species belonging to two classes e.g. Fungi Imperfecti and Ascomycete each containing four genera. Since then more fungi have been included in the list. Under the circumstances the second subgroup is not tenable as it not only contains *Madurella* but also several unrelated species of different classes. It is therefore better to retain the name mycetoma for both the type of infection without subdividing them.

Etiology Mycetoma is a disease of the exposed parts of the body specially the feet although subcutaneous tissues of other parts of the body may be infected. Most of these patients are found to walk bare footed and give history of injury such as bruise, thorn prick or introduction of wood or other foreign materials into the skin.

Organism A large number of organisms is responsible to produce the lesion. These fungi form granules of different colours namely black, white or yellowish white and red.

Organisms with black granules—*Nocardia paraguayensis*, *Madurella*, *Glenospora*, *Aspergillus*, *Penicillium*, *Cephalosporium*.

Organism with white or yellowish white granules are mainly due to infections caused by fungi of the genus *Indiella* namely *I. mansonii*, *I. reinieri* or *I. brumpti*. Fungi of this group belong to the class Fungi Imperfecti and have not yet been

cultivated *A. madurai*, *N. brasiliensis* and *A. asteroides* also produce similar granules. Fungi of the Ascomycetes group which produce white mycetoma are *Allescheria boydii* (imperfect form is *Monosporium apiospermum*) *Sterigmatocystis nidulans*.

Organisms with red granules—*Nocardia pelletieri* and *Aspergillus* species.

Signs and symptoms The lesion appears after an incubation period of months and years. It is characterised by pain and swelling of the part with multiple sinuses and spindle shaped deformity of the foot developing after several years (Fig 2). Granules of various sizes and different colours e.g. black, white or red are discharged from these sinuses with sero purulent matter. The infection spreads to the adjacent tissues forming nodules which soften and form abscesses which burst leaving multiple openings of sinuses. In some cases there is destruction of the underlying bones. The condition often remains localised without metastasis.

Pathology The granules consist of a central mass of mycelial elements forming sclerotes. These elements are arranged in a system having a central mass of mycelial threads or debris from which radiating hyphae show a palmate or fan like arrangement. Several of these may be arranged on a common base in an imbricated concentric or mulberry fashion. The size of the granules vary from 1 to 2 mm and individual granules often coalesce to form larger aggregations of several millimeters in diameter. They may be soft and cheesy or hard and brittle. Microscopically the grains consist of 3 zones: (a) central zone of mycelial reticulum and pigment granules; (b) intermediate zone consisting of irregularly amorphous and deeply pigmented substance; (c) outer zone which is refractile and gives a typical palm leaf or ray like appearance.



Fig. 2.—Mycetoma. (a) Left foot and (b) right foot of a patient.

Histopathology In a histological section a granule is seen to consist of entangled mycelia embedded in a mass of polymorphonuclear neutrophils. Surrounding this there is a layer of granulation tissue with giant cells infiltrating round cells and fat laden macrophages enclosed by a fibrous capsule.

Cultures Cultures are obtained by inoculating blood agar plates which are incubated at 37°C. Sabouraud's glucose agar slants are also inoculated and incubated at room temperature. Anaerobic cultures are necessary in case of *A. boydii*.

Diagnosis Clinically multiple sinuses with tumefied foot is very characteristic. Granules expressed from the sinuses can be crushed under a coverslip. They show lobulated masses surrounded by pus cells. The masses contain tangled mycelium which are fine in case of actinomycetes and *Nocardia* infections. Clubs may be present in certain cases. Gram stain shows short branching forms or coccoid elements which are Gram positive. In Maduromycoses and other cases the granules are treated with sodium sulphide solution till they are soft. When soft they can be pressed under the coverslip and spread into a thin layer to show fungi which are larger in dimension.

Treatment Amputation of the foot is the only successful treatment owing to the fact that there is a high mortality rate in cases of Madura foot caused specially by *Nocardia*.

SUPERFICIAL NOCARDIOSIS OF THE SKIN

[Caused by *Nocardia keratolytica* (Acton and McGuire) Dey 1957]

Definition This is a keratolytic condition of the hands and feet caused by *Nocardia keratolytica* characterised by keratolysis of the epidermis of the palms and soles and other lesions variously described as pitted sole, mango toe, cracked heel, paronychia, onychomycosis and vesicular eruptions.

Etiology The disease is caused by *N. keratolytica* enormously present on the damp and manured soil containing organic matters. The disease is common in cultivators and gardeners who often walk bare footed. It is also common amongst housewives and maid servants who are in the habit of handling mud with their hands kept moist for a long time. The disease is generally seen during the monsoon months in India. It has also been found to occur in Europeans on coastal vessels of India as they have acquired the habit of walking bare footed on the deck.

Mycology *Direct examination* Scraping from the margin of the pit, sulci or top of the vesicle is soaked in normal saline to soften it, teased and placed on a slide with a drop of McGuire's stain which is allowed to act for 2 to 5 minutes and then mopped off with a piece of filter paper. It is then mounted with a drop of neutral glycerine under a coverslip. A little pressure on the coverslip will flatten out the epidermis. It is then examined with 1/12 oil immersion objective and X10 ocular. The hyphae are seen to consist of fine segmented branching filaments. The oldest hyphae are very closely segmented and give a beaded appearance like the chain of streptococci (Pl III fig 2). Conidia may be seen at the end of some hyphae.

Culture The diseased area is well washed with soap and water to remove all dirt and then swabbed with 75 per cent alcohol. The tissue from the margin of the lesion is obtained and soaked for about 2 hours in normal saline to soften it. The material is then teased with a pair of needles into small strips and treated with absolute alcohol for one minute and these strips are planted directly into the Norris medium at pH 7.4.

Norris medium

Soluble starch	2 gm
Dipotassium phosphate	0.5 gm
Hydrated magnesium sulphate	0.2 gm
Calcium chloride	0.05 gm
Ferric chloride	0.01 gm
Sodium nitrite	0.05 gm
Asparagin	0.05 gm
Agar	20.0 gm
Water	1000.0 c.c.

Plates prepared from this medium are inoculated with small bits of infected tissue and incubated at a temperature of 25 to 35 C or room temperature. This synthetic medium inhibits the growth of most of the secondary organisms. In four days the primary culture shows small raised pink colonies (Pl III fig 3). The colonies become larger and deeper in colour with the age of the culture and still later develop into flat moist colonies with areas of darker or black tint. In Sabouraud's medium subcultures show the same character of growth (Pl III figs 4 and 5). On blood agar the culture shows a zone of haemolysis.

Biochemical reaction. Sugar or glycerol is not fermented. Milk becomes slightly acid and clotted.

Morphology. The hyphae are about 0.8μ in diameter. They are segmented, branched and show conidia (1 to 1.5μ in diameter) which may be single or in cluster. Some mycelia may have clubbed ends due to swelling of the end of the hyphae. There are often intercalary chlamydospores with thickened walls.



Fig. 3—The characteristic pitted scale of the disease.

Signs and symptoms. The disease is characterized by pitted appearance of the affected parts. But in this disease persistent cracking and scaling take place. According to the type of the lesion several clinical types have been described by Anton and McGuire (1930, 1931).

Callosi or sieve-like pitted scale (Fig. 3) shows small multiple pitted area with moth-eaten appearance (Pl IV fig 1). They may show similar pitting on the palms of the hand (Pl IV fig 7).

Plate III
COMMON PATHOGENIC NOCARDIA

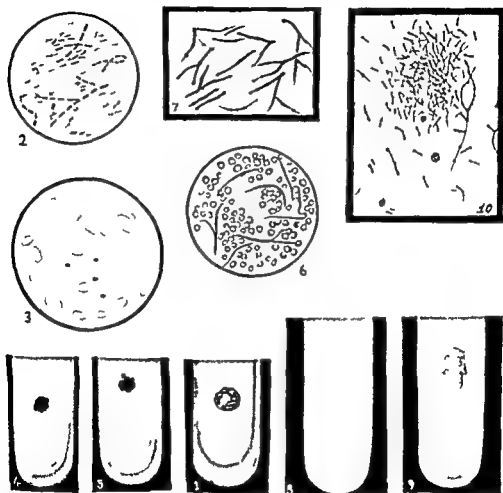


Fig (1) Growth of *Nocardia madurae* (Sabouraud's glucose agar)

Fig (2) Skin scraping showing *Nocardia keratolytica* bacillary and coccid forms

Fig (3) Primary culture of *N. keratolytica* on Norris agar plate

Fig (4) Early colonies of *N. keratolytica* (Sabouraud's glucose agar)

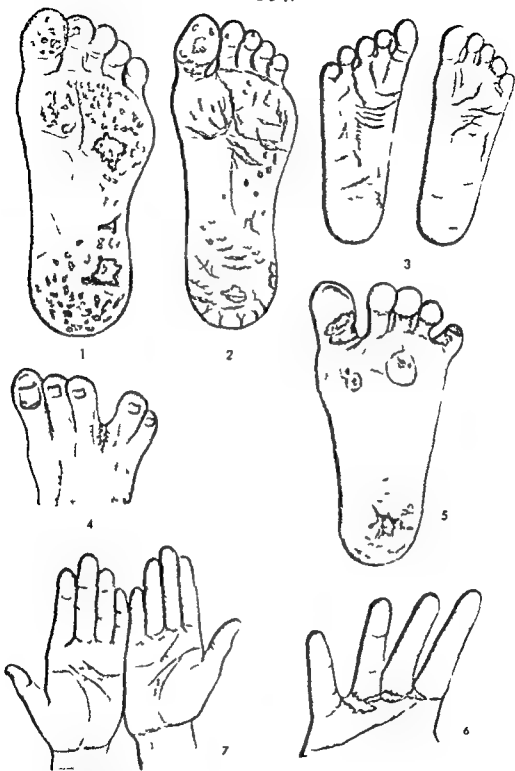
Fig (5) Old colonies of *N. keratolytica* showing black colouration at the periphery

Fig (6) Mycelia and spores with terminal clubs in culture of *N. keratolytica*

Fig (7) *Nocardia minutissima* in scales (McGuire's stain)

Figs (8) and (9) *Nocardia tenuis* in culture

Fig (10) *N. tenuis* showing metachromatic granules and branching in places (McGuire's stain)



- Fig. (1) Sole of the foot showing a vesicle-like appearance of the skin.
 Fig. (2) Cracks and fissures of the skin at the margin of the foot and heel.
 Fig. (3) Hypertrophic lesions on the sole of the foot with cracks and fissures.
 Fig. (4) A condition between the toes (interdigital) and a ulcer at the base of the nail.
 Fig. (5) A deep ulcer on the great and little toes as a result of infection of the nail.
 Fig. (6) A deep area of keratosis seen at the heel.
 Fig. (7) A ulcer on the palm of the hand as a result of extension of intertrigo.
 Fig. (8) A ulcer on the palm of the hand (a) and (b) to (c) to (d).

Phata This is a condition of cracked heel and fissures appearing at the margin of the sole (Pl IV figs 2 and 3) Hyperkeratosis is often associated with it (Pl IV fig 3)

Haja or intertrigo (Pl IV Figs 4 5 and 6) is an interdigital affection of the webs of the toes and fingers. The condition somewhat resembles mingo toe caused by ringworm fungi (Pl IV Fig 4). The infection starts as an intertriginous lesion on the webs of fingers and toes and extends to the palmar or the plantar surfaces as gyrate areas of keratolysis (Pl IV Figs 5 and 6). The ulceration generally progresses to a considerable depth producing in some cases spontaneous amputation of the toe.

Paronychia The fungus has been found to produce inflammation around the soft tissues of the nail of the hands and feet and sometimes the base of the nail is involved showing ulceration (Pl IV fig 4). The nail plate when affected may give a moth eaten or pitted appearance with darkening of the nail.

Pompholyx Vesicles may appear on the palm instep or sole. They leave pitted scars on the affected areas of palms (Pl IV fig 7) and soles.

Differential diagnosis Phata or fissures at the margin of the foot and heel and Chalusi or pitted sole are easily diagnosed by the typical appearance of the lesion.

Haja (intertrigo) or interdigital erosion should be differentiated from those produced by ringworm fungi. In the latter microscopic examination of scrapings should show numerous ringworm fungi under the high power objective. When the examinations of scale is negative for ringworm fungi one should consider investigation for *N. keratolytica*. Interdigital affections of the webs of the fingers show a sodden worm eaten appearance which spreads on the palm in the contiguous parts. The deep fissures of mingo toe are more characteristic of *N. keratolytica* infection than those of ringworm. This is due to the marked keratolytic action of the fungus.

Hypertrophic lesions of the palm and sole must be differentiated from tylosis, keratoderma of palms and soles caused by syphilis and yaws and hyperkeratosis caused by ringworm. Localised keratolysed areas caused by *N. keratolytica* often simulate perforating ulcers of leprosy and it should be differentiated from the latter condition.

Paronychia caused by *N. keratolytica* should be differentiated from similar lesions produced by *Candida albicans*. In the former condition the base of the nail shows keratolysis and pitting of the nails is often associated with interdigital affections but in the latter the margin of the nail shows bolster like swelling.

Nails affected by *N. keratolytica* are like those affected by ringworm fungi but in ringworm of the nail a positive finding of the ringworm fungus clinches the diagnosis whereas in the affection caused by *N. keratolytica* fine mycelia can be demonstrated with McGuire's stain under 1/12 oil immersion objective. Pitting of the nail is found in this as well as in psoriasis therefore psoriasis must be excluded in these cases by exclusion of the lesion in other parts of the body.

Prophylaxis The practice of walking bare footed should be avoided. In the case of cultivators who are inevitably exposed to the infection the prophylactic application of Lotio formalin et glycerine* is advantageous.

Treatment For *chalmi* The application of Lotio formalin et glycerine* is useful. For other conditions a 2% solution of gentian violet in 10% alcohol is quite satisfactory. Recently Lotio triple dye* has been used. It is quite as effective as in mango toe and other interdigital affections. In paronychia the lotion should be applied well inside the margin of the nail.

ERYTHRASMA

[The causative organism is *Nocardia minutissima* (Buchardt) Verdun 1912]

Erythrasma is a superficial infection of the skin of the moist parts of the body viz., the axilla (Fig 4) groin fold of the breast and other intertriginous regions caused by *N. minutissima*. It is characterised by brownish desquamation with furfureaceous scales showing maceration and excoriation of the affected part. The affected skin shows a serpiginous and erythematous margin. It is common in the tropics and seen in obese people sweating profusely.



Fig. 4—Erythrasma on the axilla

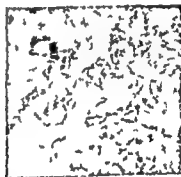


Fig. 5—*Nocardia minutissima* in axilla
(McGuire's stain)



Fig. 6—Trichomycosis axillaris (axillary hair)

See appendix

Culture—The organism can be easily cultivated on blood agar and the growth occurs after 24 to 48 hours as opaque white pinhead like colonies. They appear as fine filaments with metachromatic granules. The diameter of the organism is less than 1μ . Branching of the filament is rare and smaller bacillary or coccoid forms are occasionally seen.

Laboratory diagnosis is easily made by direct examination of the scales under the microscope by McGuire's stain. For this the part is scraped with a Paget's knife and the scales are collected between a pair of sterile slides. The scales are then treated with a drop of ether alcohol mixture and a coverslip preparation is made with the stain. The wet preparation is examined under the $1/1$. oil immersion objective. A minutissima is seen on the scales as fine filaments with metachromatic granules (Pl III Fig 7 and Fig 5). Culture confirms the finding.

Treatment—The part is kept dry by dusting Pulv. sulphur et camphor*. Lotio triple dye* may be painted once at night and this will stop the acute oozing condition within a few days. A 10 per cent solution of sodium propionate in alcohol may be sponged twice daily.

TRICHOMYCOSIS NODOSA

Nodosities of the hair is caused by *Acordia tenuis* and characterised by irregular deposit of small excrescences along the hair shaft which can be better felt than seen. They are black, red or yellow in colour. The disease is seen in the axillary or pubic hair. The affected hair does not become brittle. The disease is a chronic one and does not cause any inconvenience except that it often colours the sweat which invariably stains the under garments.

The cortex of the hair shows development of concretion in the middle or subterminal parts of the hair (Fig 6). In the parasitic life the fungus often appears in the shape of bacillary or coccobacillary form packed closely and embedded in an amorphous cementing substance.

Laboratory diagnosis Preparations from the hair often shows coccobacillary forms. The organism can be cultivated in suitable media avoiding secondary contaminants like Micrococci (*Rhodococcus*, *Nigrocooccus*) always associated with it. When the material is treated sufficiently with ether alcohol mixture contamination can be avoided. They may be cultivated in asetic fluid agar, Dorset's egg media or Loeffler's media. They produce semi translucent granular mammillated colonies which are cream coloured (Pl III Figs 8 and 9). A pure culture should not show red or black colour due to the growth of Micrococci.

The organism is 0.2 to 0.6μ in breadth and 2 to 10μ in length with occasional branching (Pl III Fig 10). They are Gram positive non acidfast coccobacillary forms are also seen.

Treatment The hair should be clipped short and should not be allowed to grow long. Dusting of the affected region with Pulv. sulphur et camphor* will keep the part dry and will stop the growth of the fungus.

CHAPTER V

SUBORDER BLASTOSPORINI AT Vuillemin 1911

Order Thallosporales Vuillemin 1910

Blastosporineae are hyphomycetes more than $1\ \mu$ in diameter and reproduce by blastospores i.e. by budding of cells. The buds are spherical or oval in shape and continue to multiply without any resting phase.

Family Cryptococcaceae Kuntitz 1833. The cells are isolated, reproduce by budding or pseudomycelia. They may or may not ferment sugars.

Subfamily Cryptococcoideae. Reproduce by budding and a few pseudomycelia. Mycelia may form in culture. Sugar is not fermented.

Genus *Cryptococcus*—Reproduce exclusively by budding in tissue and culture e.g. *C. neoformans*.

Genus *Pityrosporum*—Reproduce by budding and pseudomycelia, found in various distorted forms in tissue, difficult to grow in culture e.g. *P. ovale*.

Genus *Blastomyces*—Budding forms are seen in the tissue and in culture at 37°C and mycelia with conidia develop at room temperature e.g. *B. dermatitidis*, *B. brasiliensis*.

Genus *Coccidioides*—Spherules and sporangia like structures in tissue, mycelia and chlamydospores are seen in culture e.g. *C. immitis*.

Genus *Histoplasma*—Budding forms are seen in the tissue and in culture at 37°C , mycelial form, small conidia and prickled chlamydospores are seen in culture at room temperature e.g. *H. capsulatum*.

Genus *Phialophora*—Budding singly or in pairs in tissue and mycelial form and spores in culture e.g. *P. verrucosa*.

Subfamily Candidoideae—Yeasts with true mycelia and blastospores*.

Genus *Candida*—Mycelial and budding forms are seen e.g. *C. albicans*.

Genus *Malassezia*—Mycelia and blastospores with double wall e.g. *M. furfur*.

BLASTOMYCETES (Yeast like fungi)

Yeasts are unicellular organisms of the vegetable kingdom, often oval in shape. They reproduce predominantly but not exclusively by budding. In simple budding, the cell wall shows protrusion through which protoplasm bulges out. The bud remains attached to the mother cell so long as it is not mature. After maturation, the bud assumes the character of a mature cell and eventually becomes detached.

* It is interesting to note that the genera *Tetraporina* and *Geotrichum* are included in the Candidaceae but in this book they have been put under the Blastosporales, not under Blastomycetes.

Cytology Yeast cells consist of protoplasm with a cell wall. The cell contains a single nucleus and numerous granules and vacuoles representing reserved food matters. The cell wall is thin when young but as the cell becomes old the cell wall becomes thicker and the cell becomes filled up with granules and vacuoles. Many of these granules are volutin or metachromatic granules.

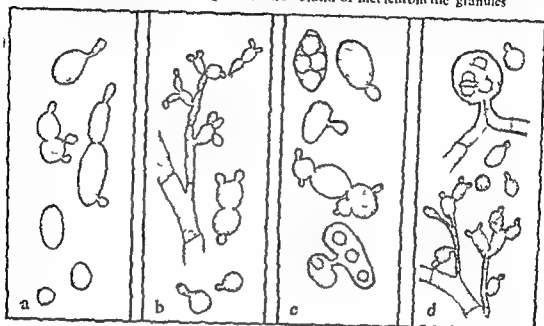


Fig 7—(a) *Cryptococcus* (b) *Candida* (c) *Saccharomyces* (d) *Endomycopsis*

Reproduction of yeasts : According to the mode of reproduction yeasts are divided into sporogenous and asporogenous yeasts. The sporogenous yeasts undergo sexual reproduction and belong to the class Ascomycetes comprising two important genera namely *Saccharomyces* and *Endomycopsis* under the order Endomycetales. The former reproduces vegetatively by budding only but the latter reproduces both by budding and mycelia. Asporogenous yeasts do not show any sexual reproduction and so belong to Fungi Imperfecti. They reproduce only by budding as in the genus *Cryptococcus* but in the genus *Candida* they reproduce both by budding and mycelial forms. The reproductions of sporogenous and asporogenous yeasts are shown in Fig 7.

CRYPTOCOCCOSIS

Synonyms Torulosis, European histomycosis, *Torula meningitis*.

Definition It is a rare disease caused by *Cryptococcus neoformans* with a predilection to produce lesions simulating tuberculous meningitis, brain abscess or brain tumour. It may cause granulomatous lesions of the skin, lung and other tissues of the body. Respiratory tract is the probable portal of entry.

History Busse (1894-95) in Germany isolated a yeast from the localized subperiosteal infection of tibia which subsequently became generalized and the patient died of multiple lesions of the skin and viscera. Hansemann (1905)

isolated a yeast like organism by lumbar puncture of a suspected case of tuberculous meningitis Stoddart and Cutler (1916) in USA reported two cases presenting signs and symptoms of cerebral tumours caused by yeasts and the organism was called *Torula histolytica* Vuillemin (1898) placed the fungus in the genus *Cryptococcus* and now the accepted name for this organism is *Cryptococcus neoformans* *C. neoformans* and *T. histolytica* causing cryptococcosis in America and Europe respectively were shown to be identical by Benham (1935)

Etiology Males are more frequently affected than the females Most of the cases are between the ages of forty and sixty The causative organism is *C. neoformans* a budding fungus which does not show formation of mycelium or sexual spores and belongs to the class Fungi Imperfecti

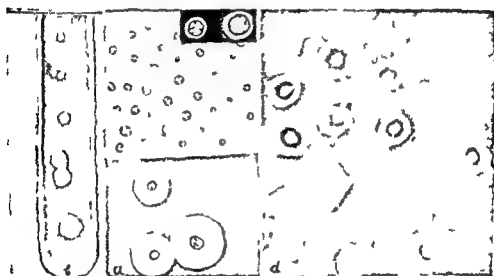


Fig 8—(a) Cerebrospinal fluid showing yeasts of *C. neoformans* in Indian ink preparation (b) Culture on glucose agar (c) Yeast form in culture (Salmonella preparation) (d) Budding forms seen in section of the brain

Mycology *C. neoformans* may be found in the sputum pus cerebrospinal fluid in subjects suffering from cryptococcosis The fungus in the tissue is a single budding cell ovoid or spherical measuring 5 to 10 μ in diameter They are surrounded by a clear halo representing a wide gelatinous refractile capsule The capsule is better demonstrated in the exudate or culture under a coverslip preparation with Indian ink (Fig 8a) In the tissue it may be stained with methylene blue Gram Weigert haematoxylin and eosin or van Gieson's stains Yeast cells may show marked variations in size The cytoplasm which is refractile stains poorly In the tissue there is a clear zone of gelatinous material round the organism (Fig 8d) and this finding led to name the parasite *Torula histolytica* The parasite is Gram positive when young but Gram amphophil when old

Culture—The organism is an aerobe and grows very well in all laboratory media preferably in blood agar. The organism can be isolated from cerebrospinal fluid pus or other exudates and occasionally from blood. To isolate from contaminated material 0.3 per cent lactic acid is added to the medium and growth of the secondary organisms is thus inhibited. All material should be cultured on blood agar and glucose agar at 37°C and on Sabouraud's glucose agar at room temperature. The growth has a tendency to dimorphism. On glucose agar incubated at 37°C small mucoid colonies develop cream to brown in colour (Fig 5b). Microscopically they show capsule formation. On Sabouraud's glucose agar at room temperature the organism grows slowly producing white wrinkled granular colonies. Microscopically yeast forms are seen (Fig 5c) and rarely germ tubes in the form of pseudomycelia. On continued subculture shining mucoid cream to brown coloured colonies develop. Capsules are best demonstrated in Indian ink preparations.

Biochemical reactions are too variable and are not useful for diagnosis.

Animal inoculation Mice and rats are susceptible but guinea pigs and rabbits are less susceptible. By animal inoculation the pathogenicity of the fungus can be determined and thus a pathogenic strain can be distinguished from a nonpathogenic one by animal inoculation.

Symptoms Occasionally localised lesions may appear in the skin, mucous membrane, lymph nodes but the organism commonly affects lungs and central nervous system. In the skin the primary lesions are acneiform papules, granulomatous lesions, punched out ulcers or deep seated myxomatous tumour or abscess. Many cutaneous cases may show generalised infection with involvement of lung and central nervous system. Lymphatic involvement may simulate Hodgkin's disease. The pulmonary involvement produces symptoms mistaken for tuberculosis or neoplasm. Central nervous system is seen to be affected in majority of cases and symptoms may resemble those of encephalitis or acute meningitis of bacterial origin like tuberculous meningitis; nevertheless they may appear as abscess or tumour of the brain. The pressure of the cerebrospinal fluid is increased characterised by papilloedema and there is increase of albumen and globulin in the cerebrospinal fluid. The cell count of the cerebrospinal fluid is high and chloride and sugar contents are low. Death occurs in coma and respiratory failure.

Pathology In the central nervous system the organism often produces condition of leptomeningitis. In the brain a chronic inflammatory process may produce cysts which contain a gelatinous substance due to abundance of capsular material produced by the organism. Tubercle like structures may be seen along the blood vessel with thick exudate at the base of the brain resembling that of tuberculous meningitis.

Histopathology In sections from the biopsy material stained with hematoxylin and eosin the budding cells are seen surrounded by capsular substance (Fig 5d). The fungus may be free in macrophages with thick cell inflammatory cell reaction.

pseudotubercles may form containing epithelioid cells giant cells and lymphocytes. The centre may be either necrosed or hyalinised in some cases.

Sputum pus gelatinous material from the cyst and the centrifuged deposit of cerebrospinal fluid contain the organism which can be demonstrated by a saline or Indian ink preparation.

Immunity All attempts to demonstrate antibody response in patients suffering from cryptococcosis have given negative results. It was shown by Benham (1935) that the encapsulated fungus did not produce any circulating antibody when injected into animals but high titre of circulating antibodies were obtained when the thick capsule was removed or the gelatinous material was injected into animals.

The extract of the organism shows specific cutaneous reaction but this requires further confirmation.

Laboratory diagnosis Lesions of the skin or lymph glands are diagnosed by biopsy and culture from the infective material. Pulmonary lesions must be differentiated from other mycotic infections and tuberculosis. Presence of *C. neoformans* is characteristic and can be demonstrated by positive cultures. In meningitis the cerebrospinal fluid should be examined as a routine examination for the presence of *C. neoformans* by direct examination and culture.

Prognosis Localised lesions in the skin and subcutaneous tissues may subside and heal in a few months but the systemic torulosis is always fatal.

Treatment Localised cutaneous lesions may be removed surgically and cauterized followed by X ray therapy. Sulphonamides may be tried in adequate doses to maintain the blood concentration between 10 and 12 mg per cent. The organism is sensitive to penicillin but as it does not reach the central nervous system it may be administered intrathecally in cases of involvement of the central nervous system.

PITYROSPORUM OVALE Kile and Engman 1938

Synonyms : Flask bacillus of Unna *Malassezia ovalis* (Bizzozzero 1884) Panja 1927 *Pityrosporon* of Malassez.

This is a yeast like organism first described by Malassez in 1874. The organism is also called bottle bacillus of Unna found in the dandruff or scraping from the scalp. These are ovoid or spherical cells with budding on a broad base and often appear to be flask or bottle shaped (Fig 9). The cells vary from 2 to 10 μ in diameter.

Culture Culture of *P. ovale* is one of the problems for dermatologists as it is difficult to cultivate them. Cultures do not give uniform results and failure is often a rule than an exception. Failures are often due to contaminations. Successful cultures have been obtained by Panja (1927) in cent per cent of cases by MacLeod and Dowling (1928) Ota and Huang (1933) Benham (1939) and Emmons (1940) and others. Moore claimed to have cultivated *P. ovale* in wort agar (Difco) and after three or four days subcultures were obtained in other media from the primary growth. He reported success in about 10 per cent of cases only.

Method of Panya : This gives uniformly good results in all the cases provided suitable media and proper precautions are employed. Scales are obtained from the scalp by scraping with the help of a scalpel and the material is collected between a pair of sterile slides. The scales are picked up with a needle put in a watch glass containing sterile normal saline and washed for some time. Small bits of scales are then picked up with the help of a platinum loop and transferred to the upper drier part of Petroff's medium with 0.004 per cent gentian violet*. Four or five inocula may be made in each tube and 3 or 4 tubes should be inoculated at a time. The tubes are incubated at 37°C. The growth is visible in two or three days time. Two types of growths are seen in the primary culture —

(1) Chalky growth specially seen on the dry part of the above medium near the tail end of the slope. The chalky surface gradually extends at the periphery by fine concentric rings. The centre of the growth often forms a pyramidal mass with a ring at the top resembling a truncated cone.

(2) On the moist part of the medium bead like masses develop often mammillated with a pinkish colour which may develop further into orange colour after a few days.



FIG. 9.—Bottle beneath in seal 370



FIG. 10.—Bottle beneath in seal 370

Subcultures on glucose agar or Sabouraud's glucose agar The growth is appreciable in 2 or 3 days time as a crenated mass with irregular spike like projections on the surface or a star like growth with distinct radial striations. They become mammillated with radial striations in different directions. The striations are best observed in transmitted light. Growths on glucose or glycerine agar are scanty but if sterile olive oil is spread on the surface of the medium the colonies assume large, thick, creamy characters in 24 hours. (See butter oleic acid or linoline may also be used instead of olive oil. The oil is autoclaved

in a test tube. A few drops of the sterile oil are put into the media tubes and spread on the surface of the slope by tilting the tube. The excess of the oil is allowed to settle at the bottom. The medium is then inoculated with the culture of *P. ovale*. The colonies appear as opaque creamy large colonies like those of yeasts. The growth is more profuse near the bottom of the slope. To maintain profuse growth the oil is to be spread on the surface of the medium by tilting the tube once a day. Microscopically they show budding forms the majority being 5 to 10 μ in diameter (Fig 10).

Moore's method Wort agar medium (Bacto) is used for the culture of *P. ovale*. Growth appears on the fourth day after inoculation. The growth is slow and colonies are compact, smooth and glistening. For subculture maltose dextrose or peptone agar is used. * Wheat germ oil or butter is added to this medium as it has been found that the organism requires a fatty environment. According to Moore colonies of *P. ovale* become 2 cm in diameter after forty days. They are cushion shaped with radiating ridges at the periphery and the surface is rough with small excrescences. The colour is dull and varies from light ochraceous salmon to pinkish buff.

Microscopically cells are 3 to 15 μ in diameter the majority being 4 to 5 μ . Budding cells are present resembling the shape of a flask or gourd. The larger old cells are encapsulated with thick gelatinous coating.

Growth factors of *P. ovale* have been worked out by Rhoda W. Benham. She has shown that the fungus is lipophilic and thiamine and oxalacetic acid act as growth factors. Thiamine causes increase of the growth when added to a basal medium with asparagin or ammonium chlorid as a nitrogen source. The growth is much better with asparagin than with ammonium chloride. Ethyl oxalacetate can replace thiamine. Again ethyl oxalacetate and ammonium chloride can be substituted for asparagin and thiamine in the cultivation of *P. ovale*.

Animal inoculation Inoculation of animals and human subjects with *P. ovale* has been reported to be successful by Moore et al (1926) as it produced clinical and pathological picture (dermatitis showing erythema or brown scalliness) resembling seborrhoeic dermatitis. But it has been frequently noticed that dermatitis of similar type may be produced by application of yeasts and yeast like organisms on the abraded skin.

SEBORRHOEIC DERMATITIS

Synonyms Pityriasis capitis 'Seborrhoea' (Hebra) Hyperidrosis oleosa (Unna) Flux sebacea (Rayer) etc

Definition The condition is supposed to be caused by infection of the scalp by *P. ovale* which grows on the surface layers of the epidermis and produces a furfuraceous desquamation. The infection of the epidermis is predisposed by excessive oilness of the skin as a result of overactivity of the sebaceous glands.

Etiology During the first three years of life the soil is suitable for implantation of *P. ovale*. According to Acton the cause of seborrhoea is as follows —

Primatively the embryonic skin consists of two layers (a) basal layer of columnar cells and (b) Rauber's layer consisting of swollen cells which do not keratinise till birth. This is due to formation of some fatty substances or vernix caseosa which acts as a water proof coating preventing maceration of the skin by liquor amnii. It is this superficial layer of the skin where *P. ovale* first infects during the first three years of life. After birth presence of remnants of vernix caseosa can also favour the growth of this organism. The infection primarily affects the scalp and then spreads on to the face and extremities. In the adult life the infection occurs in relation to sebaceous glands. To understand the sites of infection distribution of sebaceous glands of the skin is to be understood. Sebaceous glands are of two main types: (a) Sebaceous glands which are not connected with hair as an appendage. They are large or racemose in character and (i) connected with sexual organs as in the scrotum, labia prepuce and axilla. They produce volatile fatty oils imparting a distinct odour to the individual or species (ii) Sebaceous glands connected with lubrication of various orifices of the body e.g. ceruminous glands of the ear, Meibomian glands of the eyelids. In these areas *P. ovale* may cause boils of the external auditory meatus, styes, blepharitis, retention cysts etc. (b) Pilosebaceous glands occur as appendages at the root of the hair follicles and found in places where hair is present. These are further differentiated into three varieties: (i) Sebaceous glands that are at the root of long hairs. These are single, saccular or bilobed. i.e. long hairs have small glands. (ii) Sebaceous glands at the root of lanugo hairs are large and racemose in character. (iii) Multilobed large racemose glands are present in areas where glands have lost the relationship to the hairs. They are mostly seen in the forehead, nasolabial folds, back of the neck, mid thoracic and interscapular regions. There is a minimum amount of subcutaneous fat in these regions. These are the sites where seborrhoeic dermatitis is seen.

Physiological overactivity of the sebaceous gland occurs during the first three months of life and at puberty. Only condition of the skin favours the growth of *P. ovale*. According to Sabouraud greasy skin is not essential for seborrhoea but the moist condition which predisposes seborrhoea is essential and is due to serum and not sebum. At puberty along with sex glands sebaceous glands become more active.

Hereditas plays an important part in determining the number and size of sebaceous glands. Certain individuals are predisposed to this condition by virtue of inheritance of the skin containing numerous sebaceous glands.

Sex Baldness is comparatively rare in women and this might be due to the influence of different gonadal hormones in males and females.

Endocrine dysfunction The condition of seborrhoea is common at the advent of puberty when there is an imbalance of endocrine glands, specially the gonads. At that time the sebaceous glands become hypertrophic and more active.

producing a condition of seborrhoeic state. The male gonad or androgen is particularly responsible for this condition and helps in the formation of a suitable soil for the growth of *P. ovale*.

Mode of life A sedentary city life with lack of exercise predisposes to seborrhoea and exposure to the sun often increases the condition.

Food Diet rich in carbohydrate or fat producing lack of digestion predisposes to seborrhoea. It has been observed that deficiency of vitamin B-complex which makes the patient sensitive to sunlight sets up keratosis and exfoliation of the naso labial folds.

Relative acidosis Urine is highly acid in seborrhoeic patients and the alkali tolerance of the patient is high as large doses of alkalies are necessary to make the urine alkaline.

Age Seborrhoea commences in early life shortly after birth due to extraneous infection of *P. ovale* which is the determining factor. A few days after birth vernix caseosa is shed off and the superficial layer of the skin begins to keratinise. Scalp with the presence of vernix caseosa forms a good soil for the growth of *P. ovale*. Once the infection occurs the disease progresses upto the age of three years after which the seborrhoeic manifestations disappear temporarily. At puberty it reappears and continues for the rest of life. At puberty the activity of the gonads increases giving rise to a peculiar condition of the skin that predisposes to pityriasis capitis and acne. Darier has described the condition as kerosis in which the complexion of the patient is muddy and the skin is coarse greasy and thickened. Mouths of the sebaceous glands are large and patent.

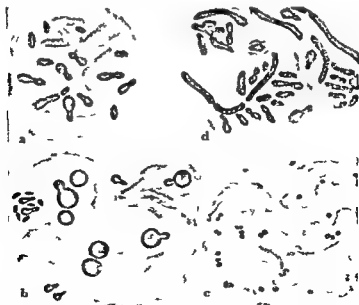


Fig 11—Botryobasidium ovale (a) antheridium (b) flask form (c) coelium (d) mycelial form

but many of them are blocked with dried up inspissated sebum forming black heads (comedones). The condition often disappears spontaneously at the age of 25 years or so but it may persist through out the whole life.

Organisms : The rôle of the organisms responsible for seborrhoeic condition is still disputed but it is a fact that *P. ovale*, *Staphylococcus albus* and *Corynebacterium acne* form the seborrhoeic triad. *P. ovale* is found in flask shaped form (bottle bacillus of Unna), coccal form (*morococcus* of Sabouraud) or rarely in the rudimentary mycelial form (Fig 11). Coccal forms are seen when the disease is spreading rapidly. *C. acne* is specially found in acne vulgaris. Experiments of MacLeod and Dowling have proved that seborrhoeic infection is a pure fungus infection with *P. ovale*. The organism grows well in subjects with moist and greasy skin.

Signs and Symptoms : Seborrhoeic lesions are best described under three age groups. The types of the lesion and their symptoms vary according to the age and the area affected.

(a) **INFANCY** *Milk crust* : This condition develops within a few days after birth if vernix caseosa is not thoroughly removed. The child becomes infected with *P. ovale* from the mother or the nurse. In this case vernix caseosa acts as an ideal culture medium for its growth. The scalp becomes inflamed and very irritable resulting in serous exudation and crust formation. The scalp is covered with a cap of hair matted with exudate mixed up with dirt and contains the infecting fungus. Impetigo is superimposed on it in many cases due to infection of the skin caused by streptococci.

Infantile eczema : There are various causes of infantile eczema but in certain cases it is due to extension of the lesion from the scalp to the skin of the forehead, cheek, behind the ears, flexures of the body, outer side of the legs. It is characterised by serous exudate due to secondary streptococcal infection producing yellow crusts like impetigo. There is an intense itching associated with it. Food allergy may maintain the condition or aggravate it. These children are often subjects of prickly heat, impetigo and boils in the later life.

Liver spots : These are localised patches of seborrhoeic dermatitis with superficial exfoliation and development of hypopigmented patches on the face and back popularly known as liver spots.

(b) **FROM PUBERTY UP TO THE AGE OF 35 YEARS** *Seborrhoea capitis* (Pityriasis sicca of Hebra, dandruff) : It is a dry scalp condition of the scalp which is very common during adolescence. Brain like scales accumulate on the scalp causing exfoliation of the outer horny layer and mild irritation of the skin.

Seborrhoea oleosa (Hebra) : This usually occurs at puberty characterised by the greasy condition of the skin containing *P. ovale*. They are the subject of acne vulgaris and seborrhoeic alopecia in later life.

Seborrhoea corporis : The condition is also called flannel rash owing to the old conception that it was due to wearing flannel garments next to the skin. The infection spreads from the scalp to the front of the chest and the trunk. There

are irregular hypopigmented circinate patches of different sizes varying from a four anna bit to that of a rupee. Lesions are seen on the face neck sternal and interscapular regions but they may spread to other parts of the body viz arms thighs & antero-crural region and perineum.

Seborrhoeic eczema This is a dry scaly condition of the neck face and eye lids but later they may show a weeping eczematous condition on the flexural aspects of the axillae elbow or knee joints.

Seborrhoeic lichen This occurs as a result of irritation or itching and is generally seen on the nape of the neck and flexures of the elbow and other regions where there is a condition of seborrhoeic eczema. This starts as a group of papular rash in patches due to hyperkeratosis of the hair follicles. It is associated with thickening of the skin on the nape of the neck with raised margin scalliness and pigmentation in seborrhoeic subjects.

(c) FROM 30 TO 45 YEARS **Seborrhoeic baldness** : During this period the infection is restricted to the scalp and involves the true pilosebaceous glands. The infection destroys the glands as well as the hair follicles producing seborrhoeic alopecia or baldness. The baldness may start from the crown and extend towards the forehead or it may start from the forehead and spread towards the crown of the head. Baldness is diffuse and complete within a few years. The whole of the forehead and vertex of the scalp is denuded of the hair the skin becomes shiny and only a little area on the side and back of the head shows thin growth of hair.

PREDISPOSITIONS Prickly heat is one of the seborrhoeic predispositions and the condition is characterised by the infection of sweat pore by staphylococci. The epidermis at the top of the vesicle shows both staphylococci and *P. ovale*. It is usually seen in persons suffering from seborrhoea of the scalp.

At the age of puberty the condition of kerosis is commonly seen. The pilosebaceous glands which undergo hypertrophy are exposed to infection by staphylococci and *P. ovale*. *C. acne* also invades the sebaceous glands when mouths of the glands are obstructed and partial anaerobiasis is produced. This is favourable for the growth of *C. acne*. The glands that are usually attacked are those of naso labial folds forehead sternal and interscapular regions. In deep seated lesions they produce scars which may subsequently form keloids. The mouths of the glands are blocked and form black heads (comedones) due to drying up of sebum mixed with dirt but in other cases retention cysts are formed.

Pathology *P. ovale* grows on the superficial layers of the epidermis and appears as yeast like budding forms. During multiplication it causes alteration in the superficial cells preventing them from perfect keratinisation. In the affected areas the scales are cast off as tiny delicate pellucid scales called dandruff. When the disease is rapidly spreading rounded coccal or rarely mycelial forms are seen. In seborrhoeic alopecia the horny layer in the hair follicle fails to keratinise and the mouth of the follicle is filled with large swollen cells. Deeper in the follicle minute coccal forms and yeast forms are seen as far down as

pilosebaceous glands which are surrounded by an inflammatory zone of leucocytes. The hair is distorted in shape and later on the hair root undergoes atrophy and subsequently the hair follicle is obliterated resulting in baldness of the scalp.

Treatment : In treating cases of seborrhoea it should be remembered that the primary site of infection is the scalp and sulphur in various forms is the drug of choice. Secondary infections are responsible for the persistence of eczematous lesions in infants.

Milk crust : In this condition prophylactic measure is very important and thorough removal of vernix caseosa should be strictly adhered to. This can be done by rubbing olive oil on the scalp followed by gentle rubbing with soap and water to remove every trace of oily matter. Should any scaldiness persist lotio resorcin diluted with equal parts of water should be applied on the scalp. Application of 0.5 per cent hydrarg oleata in olive oil is also useful. In established cases of infection sulphur salicylic ointment (15 grs of each in an ointment base) should be applied.

Infantile eczema : Treatment in these cases should be local and general. Weeping eczematous lesions on the face (eczema rubra) are best treated with lotio calamine in the weeping stage till the part dries up. When the part is dry and weeping has stopped liniment calamine with sulphur (10 grs to an ounce) should be applied thrice daily followed by application of 3 per cent resorcin ointment at night. In this primary infection of the scalp should be remembered and lotio resorcin (diluted) should be used as mentioned before. The general treatment consists of relieving constipation by administration of gray powder in doses of 1/4 to 1/6 gr according to age twice daily. Treat the coexisting food allergy by changing the milk or the diet as the case may be.

Seborrhoea capitis : In this and other seborrhoeic conditions in adults remove the scales and promote the free flow of sebum by keeping the gland orifices clean with application of spirit soap or ether soap shampoo (See appendix). The shampoo may be rubbed twice weekly. If it is rubbed more frequently the affected hairs which become brittle may break. As an antiseptic and exfoliative lotio resorcin et hydrarg perchloride is an effective application on the scalp. For seborrhoea oleosa 1 per cent oleate of mercury in olive oil is effective for cleaning the scalp. For seborrhoea of the face and body lotio sulphuris alba and Pulv sulphur et camphor should be applied alternately each twice daily. The former is a time honoured remedy for seborrhoea of the body and the latter is used in the summer when one perspires profusely. Lotio sulphuris flava is almost as efficacious as lotio sulphuris alba. On flexural eczema and other irritable conditions liniment calamine with sulphur (10 to 15 grs per ounce) is indicated along with other remedies. In seborrhoeic lichen the above treatment should be combined with application of unguentum acid salicylic on the affected part at night as an exfoliative. The patient responds very well with this treatment.

Acne vulgaris : In this condition along with the local applications general treatment is also indicated. Constipation dyspepsia in digestion should be treated.

The quantity of carbohydrate and fat in the diet is reduced. Intake of alcohol and highly spiced food are restricted. Administration of vitamin B complex, yeast marmite and liver extract are useful. All attempts should be made to improve the general health of the patient and iron should be administered as a tonic.

BLASTOMYCOSIS

Synonyms North American blastomycosis, Gilchrist's disease, Chicago disease.

Definition This is a chronic infectious disease caused by a yeast-like fungus called *Blastomyces dermatitidis* characterised by primary granulomatous lesion of the skin or lung and secondary metastatic lesions in other parts of the body.

A few doubtful cases have been reported from India.



Fig. 1—Blasting cell and p. offlat as a

History The organism was first discovered by Gilchrist (1896) in a section of skin lesion resembling tuberculosis. In a second case Gilchrist and Stokes (1898) cultivated the causative organism and named it *Blastomyces dermatitidis*. The organism was extensively studied by Ricketts (1901) who differentiated it from true yeasts and called it *Oidium dermatitidis*. Vuillemin named it *Cryptococcus gilchristi* which terminology was supported by Sartory and Castellani. It was placed in the genus *Mycoderma* by Brumpt.

Mycology **Direct examination** A coverslip preparation is made with a loopful of pus or sputum with a 10 per cent solution of potassium hydroxide. It shows characteristic round or oval thick-walled double-contoured refractile yeast-like bodies with budding 8 to 20 μ in size. The bud has a thinner wall than the mother cell. These cells are highly granular bodies and occur singly in pairs or groups and are pathognomonic of this infection (Fig. 12).

Culture Dimorphism is characteristic. Primary cultures are made on blood agar and glucose agar and incubated at 37°C. The fungus grows slowly and the culture should not be rejected as negative before one month. The colonies develop as waxy wrinkled growths. On microscopic examination

budding yeast like cells are seen resembling those in the lesion. Subcultures on Sabouraud's glucose agar at room temperature exhibit downy growths characterised by aerial mycelia which are at first white but become brownish with age (Fig 13a). Microscopically it shows mycelia with oval or round conidia 3 to 4 μ in diameter attached to the hyphae. Pyriform conidia are about 5 μ in length and borne on lateral sterigma. In old cultures chlamydospores are seen 7 to 8 μ in diameter with thickening of the wall (Fig 13b). Mycelial form may be converted into yeast form by incubating the subcultures at 37 C.



Fig 13—(a) Culture of *B. dermatitidis* on Sabouraud's agar at room temperature
(b) Culture showing mycelia bearing conidia

Animal inoculation. The mouse is the most susceptible animal; rats and rabbits are less so and guinea-pigs are the least susceptible. Any phase either the mycelial or the budding one injected into mice causes local lesion followed by visceral lesions of the liver, spleen or lungs in about 3 weeks.

Pathology. The stages of evolution are those of an infective granuloma. Minute abscesses are formed as a result of necrosis. Lesions in the skin may be in the form of a nodule, papillomatous or a gummatous ulcer but lesions in the lungs or viscera show abscess formation with ulceration. The lesions spread by continuity and contiguity. Dissemination may occur through the haematogenous route.

Histopathology. The histopathological changes are those of a granuloma. In the skin lesions acanthosis and papillomatosis are marked. There are minute intraepidermal and intradermal abscesses in which budding yeast cells are present. Interstitial and parenchymatous oedema is present in the underlying cutis where changes are essentially those of a granuloma. The abscess consists of degenerated polymorphonuclear neutrophils which are found around the organism. Beyond the zone of polymorphs there are macrophages with formation of giant cells of Langhans type containing the organism. Fibroblasts are present at the periphery. The characteristic round or oval budding cells are present in the pus and best stained by polychrome methylene blue.

Symptoms The period of incubation is not definitely known but it varies from one to two weeks. Clinically blastomycosis is divided into

(a) **Primary cutaneous blastomycosis** In this the primary lesion is in the skin and secondary systemic involvement is seen in about 33 per cent of these cases specially in the lung

(b) **Systemic blastomycosis** 95 per cent of these cases show extensive pulmonary involvement. The condition is extremely fatal. Dissemination occurs in the bone (60 per cent) liver spleen and kidney (40 per cent) central nervous system (30 per cent) and in the prostate (20 per cent)

Cutaneous blastomycosis It may manifest itself as primary papulo ulcerative lesion papillomatous or granulomatous ulcers or as subcutaneous nodules forming ulcers covered with scabs. Exposed parts of the body namely face lower part of the legs and hands are more often infected. In these cases trauma may play an important part. The centre of the lesion often heals up and the spreading margin of the lesion shows military abscesses with crust formation. When the crust is removed the granulation tissue or small sinuses are seen with seropurulent discharge containing the fungus. Cutaneous lesions secondary to systemic infection shows superficial or deep ulcers with granulation tissue and exudation of pus forming crusts. Multiple lesions may appear in the skin as a result of autoinoculation. Metastatic spread may occur from the skin to internal organs

Pulmonary blastomycosis It is usually a primary involvement but may be secondary to a focus in the skin. Systemic blastomycosis has also a tendency and affinity for metastasis in the lung. The primary infection occurs through the respiratory tract. The onset may be insidious but it may be rapid in acute cases. In these cases pain in the chest fever cough dyspnoea and expectoration of blood tinged sputum and loss of weight are characteristic. The chest signs are like those of a pulmonary abscess or massive tuberculous infection. Pulmonary blastomycosis should be suspected if discharging sinuses or subcutaneous abscesses are seen over the chest wall

Cerebrospinal blastomycosis This is usually a secondary condition and occurs as a metastatic process from a primary focus. Signs and symptoms are similar to those of cerebrospinal infection due to other mycotic organisms

Diagnosis The diagnosis depends on the demonstration of *B. dermatitidis* in the pus sputum or cerebrospinal fluid by direct microscopic examination. The material is examined with a drop of 10 per cent potassium hydroxide and budding cells with refractile capsules should be observed. Culture should also show the growth of the organism. Histopathological examination of the skin lesion will show a granulomatous lesion showing budding cells with double contour

Intradermal test with a standardised heat killed vaccine is useful in the diagnosis of the disease. The reaction is of tuberculin type 0.1 cc of the vaccine is injected intracutaneously and readings are taken after 12, 24 and 48 hours. Positive reaction shows an area of erythema of 1 cm or more in diameter after

48 hours. In highly sensitised patients a sterile abscess forms after 48 hours. Complement fixation test with suspension of the organism as antigen is also positive in these cases. Both skin test and complement fixation test should be done because when the complement fixation test is negative the skin reaction may be positive and vice versa but there are cases in which both the tests are positive. Positive skin test indicates presence of hypersensitiveness.

Prognosis. Primary cutaneous lesions may be cured if proper treatment is given early in the diseases but 92 per cent of systemic involvement become fatal. Prognosis in cerebrospinal cases is always grave. Prognosis is best in hypersensitive cases but without positive complement fixation test which indicates spread of the lesion.

Treatment. Whatever therapeutic measures are taken proper care should be taken about the general health of the patient. Sunbath, vitamins, high caloric diet are essential for the well being of the patient.

In the cutaneous type complete resection followed by cauterisation with diathermy is advisable in early cases. A full dose of Pot iodide orally upto the limit of tolerance should be supplemented by 1 to 2 gm. of Sod iodide by intravenous route. Iodide in big doses may be harmful in hypersensitive cases. Martin and Smith therefore advise desensitisation of the patient with vaccine before the iodide therapy. X ray therapy is useful in doses of 125r through 1 mm. aluminum filter for several exposures. This therapy should be administered after the patient has been desensitised with specific vaccine therapy.

Specific therapy. In systemic blastomycosis iodide treatment is combined preferably with autogenous vaccine. Otherwise a standardised vaccine may be used for this purpose. The initial dose is 0.1 c.c. and it is increased by 0.1 c.c. on every alternate day till 1 c.c. is reached. The increase of dose should depend on the absence of skin reaction. If the skin reaction is 1 cm. or more the vaccine is diluted to 1 in 100. If the skin reaction is 2 cm. or more the vaccine should be diluted 1 in 1000 and so on.

SOUTH AMERICAN BLASTOMYCOSIS

Synonyms. Piracoccidioid granuloma, Lutz Splendore de Almeida's disease.

Definition. South American blastomycosis is a chronic granulomatous ulcer of the skin and mucous membrane with enlargement of the lymph nodes and involvement of the internal organs caused by *B. brasiliensis*. The granulomatous and ulcerative lesions of the oral mucosa, enlargement of the lymph nodes and involvement of the gastrointestinal tract differentiates the condition from North American blastomycosis. The lungs are rarely affected in this condition.

History. Lutz (1904) reported the first case of pseudo-coccidioidal granuloma of the mouth and regional lymphatics occurring in Brazil. But the first case of generalised piracoccidioidosis was reported by Splendore (1919). Arantes (1920) and Fonseca (192-1929) described the organism as *Coccidioides immitis* but de Almeida (1930) compared the cultures of *C. immitis* with those of South American blastomycosis and found them to be different.

Etiology. The disease is endemic in South America particularly in Brazil but cases have been reported from Argentina, Paraguay, Peru and Venezuela. It is found in people of rural communities. Males are more commonly affected than females (10, 11). The causative fungus *Blastomyces* (*Paracoccidioides*) *brasiliensis* has been isolated from the lesion, soil and natural substrates. The infection often follows a trauma.

Mycology. *B. brasiliensis* like *B. dermatitidis* is dimorphic and both yeast and mycelial forms are seen. In the tissue, pus or culture at 37°C single or multiple budding cells are seen; the latter is diagnostic. Buds unlike those of *B. dermatitidis* are coccoid, small, measuring 1 μ or more. They are typically multiple and arranged round the mother cell. Single budding cells are 10 to 30 μ in diameter but the multiple budding cells may be as large as 60 μ (Fig. 14).

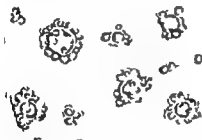


Fig. 14—Clear tertiary bulla of *B. brasiliensis* in pus.

Fig. 15—Growth of *B. brasiliensis* on Sabouraud's glucose agar at room temperature 4 weeks old.

Culture. The infective material is cultured in blood agar and Sabouraud's glucose agar and incubated at 37°C and at room temperature. On blood agar incubated at 37°C the culture is yeast-like and single and multiple budding forms are seen like those in the lesion. On Sabouraud's agar incubated at room temperature the growth is heaped up, glabrous and cerebriform like that of *Trichophyton schoenleinii*. These often show budding cells but soon the growth is covered with white aerial mycelia (Fig. 15). They show filamentous mycelial forms with small, smooth-walled, round or pyriform conidia and chlamydospores.

Animal inoculation. Mice are inoculated intraperitoneally but guinea-pigs are inoculated intraperitoneally and intratesticularly. The fungus can be studied from the pus and cultures may be obtained from the lesions after their development.

Clinical types. Several clinical types have been described: (1) Mucocutaneous type which starts from the buccal mucosa as a granulomatous ulcer and affects the oral mucous membrane and nose. (2) Lymphangitic type in which groups of lymph nodes are enlarged, namely cervical, supraclavicular and axillary lymph glands. The adenopathy is like that of Hodgkin's disease. (3) Visceral type with lesions in the intestine, liver, spleen, pancreas and other internal viscera. (4) Mixed type involving both the skin and internal viscera.

Immunity The complement fixation test is positive in spreading cases indicating a bad prognosis. It may be negative in early or treated cases. The skin test is, however, positive with paracoccidioidin in cases with hypersensitivity.

Diagnosis Infective material may be obtained from (i) pus or scraping from the ulcer on the buccal mucosa (b) Pus from a fluctuant node (c) Smear or material from biopsical nodes (d) Sputum. The material is examined in fresh state under a coverslip with 10 per cent potassium hydroxide. The fungus appears as single or multiple budding cells; the latter is diagnostic of paracoccidioidal granuloma. The organism should be cultured on artificial media. Guinea-pigs are inoculated intratesticularly with the infected material and pure cultures may be obtained from the material after development of the lesion.

Treatment Iodide has been advocated but it should not be administered without desensitisation of a hypersensitive patient. Therefore before administering iodide, skin test should be done for hypersensitivity. Recently it has been reported that South American blastomycosis responds very well to sulphapyridin in all the stages of the disease when administered over a long period. Penicillin is not effective against *B. brasiliensis* in vitro or in vivo.

COCCIDIOIDOMYCOSIS

Synonyms Coccidioidal granuloma, Posada's disease, valley fever, desert rheumatism, etc.

Definition It is a highly infectious disease caused by *Coccidioides immitis* and characterised by an acute primary benign (self limited) respiratory infection (Coccidioidomycosis) or a chronic progressive secondary disseminated granulomatous affection involving the lung, skin, subcutaneous, visceral or osseous tissues and ending fatally. The acute benign respiratory disease is known as valley fever or desert rheumatism. Primary cutaneous lesions may, however, be seen with papillomatous or fungating lesions.

History The first case of coccidioidomycosis was reported by Posada (1897) and Wernicke (1892) in Argentina affecting a Brazilian soldier. The second and third cases were seen in California by Rixford and Gilchrist (1894). They believed the parasite to be a protozoan and named it *Coccidioides immitis* in 1896. Ophuls and Moffitt (1900) cultivated this organism and found it to be a fungus with filamentous mycelia. Benign type was first described by Gifford (1936).

Geographical distribution The disease is endemic in San Joaquin valley of California and some parts of Mexico. A few authenticated cases have been reported from South America. No confirmed case has been reported from India.

Sources of infection The organism pathogenic for animals has been isolated from soil and vegetations. Natural infection has been found in the internal organs of cattle, sheep and dogs (Stiles and Davis 1942). Desert rodents have been found to be naturally infected by Emmons (1942). These rodents harbour the infection and act as an animal reservoir of the disease. The source of saprophytic fungus in the desert soil is probably maintained by the infected rodents.

Mode of infection Respiratory infection is caused by inhalation of the spores of the fungus in the dust carried by the wind. Laboratory workers may be infected by inhalation of spores from the culture. The infection is rarely acquired through the exposed surfaces of the skin.

Mycology *Direct examination* In a coverslip preparation of the material the fungus appears as a thick walled doubly contoured hyaline cyst like spherules 15 to 80 μ in diameter. They do not show budding in the tissues.



Fig 16—(a) Culture of *C. immitis* on Sabouraud glucose agar at room temperature 4 weeks old (b) *C. immitis* showing formation of arthrospores in culture ()

Culture The fungus can be grown in ordinary media or malt extract agar. Colonies appear in 4 to 6 days as slightly raised membranous disc on the surface of the slope and the growth soon penetrates through the medium. In the course of another week the growth becomes downy (Fig 16a). The downs are at first snow white but with the age of the culture they become brown in colour with abundance of chlamydospores 4 to 7 μ in diameter. In old cultures oval or round arthrospores are seen in abundance (Fig 16b). Old cultures are powdery friable and the spores easily borne by the air are infective.

The medium recommended by Dr C E Smith contains 1 per cent each of ammonium chloride and sodium acetate 0.8 per cent tribasic potassium phosphate 0.4 per cent cupric sulphate and 2 per cent agar.

Microculture Mycelia are branched and septate and the fungus forms numerous arthrospores 2 to 4 μ in diameter and separated by alternate clear and dark spaces along the course of the hypha.

Animal inoculation All laboratory animals are susceptible to coccidioidal infections. Mice are usually injected intraperitoneally but guinea pigs are injected intratesticularly with infected material or suspected cultures for positive results.

Clinical manifestations Coccidiomycosis may affect a person of any age. The incubation period is about 10 to 14 days. The primary lesion may be in the skin but it often starts in the lung by inhalation of dust containing spores. In the acute stage the lung infection resembles an attack of flu or bronchitis with slight fever, cough, headache, backache and the sputum may be blood tinged. This is known as the valley fever as it is common in San Joaquin valley.

It is also called desert fever or desert rheumatism as arthritis is often associated with it. After 8 to 15 days nodules like those of erythema nodosum and eosinophilia may develop. Recovery from the acute condition usually occurs in 3 to 6 weeks.

Primary skin lesions appear as papillomatous or verrucose lesions like those of tuberculosis of the skin.

Coccidioidal granuloma This is a progressive condition from this infection which develops in more severe cases of coccidiomycosis. In many respects it resembles pulmonary tuberculosis as reactivation of inadequately treated cases may occur analogous to reinfection of pulmonary tuberculosis. If the temperature of the valley fever lasts after the third or fourth week one should suspect the progressive form of the disease. Then new shadows appear in the lung when the skiagram is taken. As the disease progresses dissemination occurs through blood stream with metastasis in the bone, subcutaneous tissues (cold abscess or granulomatous lesions), internal organs including meninges. Generalisation may occur in any organ with the progress of the disease with pyrexia, loss of weight, night sweat and the case ends fatally in 4 or 5 years.

Immunity reaction Presence of precipitin and complement fixing antibodies has not been established definitely to help in the diagnosis. They may appear in severe infections but disappear with recovery.

Hypersensitiveness to coccidioidin develops between the second and third week of infection. For the test 0.1 cc of 1:1000 of a standard antigen is injected intradermally and this should show a positive reaction. Those who had previous skin infections may show a reaction even in a dilution of 1:10,000. Dickson and others hold that the cutaneous reaction is specific.

Treatment No special treatment is necessary in the acute stage except rest in bed till the blood sedimentation rate is normal and lungs are found to be clear by X-ray examination. Complement fixation test is negative in these cases. The prognosis is grave in progressive cases. In these cases specific vaccine therapy has been reported to be efficacious. Iodide is ineffective. X-ray therapy is helpful in causing absorption of exudate in the granulomatous process and intensive or semi-intensive doses should be used. Thymol has been found to be effective. A 33.3 per cent solution in olive oil is applied locally and orally a daily dose of 1 to 6 gm dissolved in olive oil should be administered putting it in capsules. This is administered along with meals.

HISTOPLASMOSIS

Definition This is a rare chronic disease of the reticuloendothelial system resembling kala-azar and caused by *Histoplasma capsulatum* characterised by irregular fever, hepato-splenomegaly, enlargement of the lymph nodes, anaemia and ulcerative lesions of the buccal mucosa, nose, intestine, anus and genitalia.

History The organism was first observed by Darling (1906 to 1909) in section of a spleen and liver of a patient who died of a disease resembling kala-azar. He called the organism *Histoplasma capsulatum* under the impression

Plate V
HISTOPLASMOSIS



Fig 1—Histoplasmosis on the lips, angles of the mouth and nose



Fig 2—Abscesses on the abdominal wall and nodular lesions on the groins

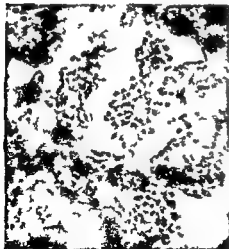


Fig 3—Section of tissue stained with Leishman's stain. P.E. cells are packed with parasites

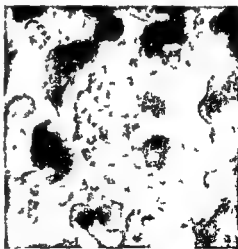


Fig 4—Smear stained with Leishman's stain. A histiocyte is packed with *H. capsulatum*

With kind permission of Drs G. Panja and S. Sen (J I M A Vol 23 25 1964)
(Courtesy of the Editor J I M A)

Plate V
HISTOPLASMOSIS
(Continued)

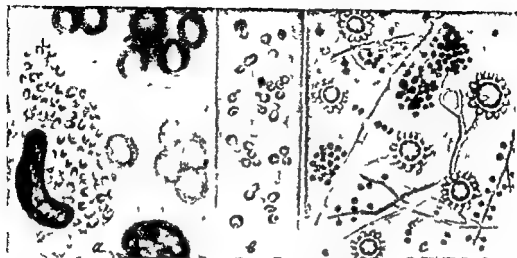


Fig 5—(a) Histiocyte laden with *H. capsulatum* (b) Yeast form in culture at 37° C. (c) Conidia and chlamydoconidia in culture



Fig 6—Culture of *H. capsulatum* in Sabouraud's glucose agar at room temperature
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that it was a protozoan. Da Rocha Lima (1912-1913) observed budding of the organism and suggested that they were related to *Cryptococcus*. Mycotic nature of the disease was finally proved by Hansmann and Schenken (1934) and DeMonbreun (1934) by culture of the organism from their cases.

Geographical distribution. Cases were reported from North and South America, South Africa, England, Philippines and Java. Recently one case has been reported by Panja (1954) in India (Pl. V Figs 1 to 4).

Source of infection. It is a naturally acquired infection in man and dog but there is no evidence that the dog is the reservoir of infection.

Portal of entry. The exact portal of entry is not known but it is most likely to be the oral route. That the infection occurs through the oral route is evidenced by the primary infection of lips, ulceration of the tongue and oral mucosa but in a few cases primary cutaneous lesions have been described. Primary infections of the lung and lymph nodes have also been reported.

Etiology. One fifth of the cases have been recorded in infants below two years but no age is exempt. Maximum number of cases are seen between 30 and 40 years of age. All races are affected but nothing is definitely known about the occupational predisposition.

Micology. *Direct examination.* Thick and thin smears of peripheral blood, sternal puncture stained with Leishman's stain show the parasite. Smears from the puncture of lymph node, spleen, liver or ulcers of the skin and mucous membrane may be similarly stained. *H. capsulatum* appears as small budding oval yeast-like cells which are single and 1 to 5 μ in diameter. They grow in the endothelial and mononuclear cells of the body (Pl. V Fig. 3). They look like *Leishmania donovani* but are slightly larger than the latter. Kinetoplasts are absent in *H. capsulatum* (Pl. V Fig. 5a).

Culture. The organism may be cultured in common laboratory media. The culture is dimorphic in character.

(a) The budding or the tissue stage is seen on blood agar incubated at 37°C (Pl. V Fig. 5b). Primary culture appears in one to two weeks to grow but the culture should not be rejected before one month. The colonies are like those of *Staphylococcus albus*.

(b) Mycelial forms are seen when they grow in Sabouraud's glucose agar or Allison's copper sulphate trypticised serum agar at room temperature. The colonies grow slowly with white downy aerial mycelia at first but later they become brown with age (Pl. V Fig. 6). They show septate hyphae bearing spores on short pedicles. The spores which measure 2.5 to 3 μ in diameter are smooth, round or pyriform. In old cultures there are thick-walled round tuberculated chlamydospores 10 to 15 μ in diameter (Pl. V Fig. 5c). The filamentous form is occasionally converted into yeast form by culturing in fresh blood agar tubes sealed (to maintain moisture) and incubated at 37°C. The yeast form can be maintained by subculturing in blood agar at frequent intervals.

Immunity reactions Precipitin or complement fixing antibodies could not be demonstrated in blood. A delayed tuberculin like reaction becomes positive with histoplasmin in 24 to 48 hours. Many workers have shown a high correlation between pulmonary calcification in nontuberculous cases and positive skin test to histoplasmin. A large number of such cases have been detected in certain areas of United States indicating a benign type of endemic histoplasmosis in those areas.

Animal inoculation Mice guinea pigs and dogs can be experimentally infected with the cultures of *H. capsulatum*.

Symptoms *H. capsulatum* causes infection of the reticuloendothelial system. The symptoms simulate those of kala-azar namely pyrexia of long duration, hepatomegaly, splenomegaly, anaemia, leucopenia and asthenia. Enlargement of lymph nodes may resemble lymphadenopathy of lymphosarcoma or Hodgkin's disease. There may be ulceration of the lip, tongue, oral mucosa, nose, anus or genitalia. Secondary pulmonary involvement may simulate one of miliary tuberculosis. Ulceration of the skin has been reported. The disease usually lasts for several months. The mortality rate is high and the condition is often diagnosed in an autopsy.

Laboratory diagnosis The parasite is found in the smear from peripheral blood, sputum, urine, ulcers or aspirated material from lymph nodes, sternum, spleen, etc. The smear should be examined under 1/12 oil immersion objective after staining by Giemsa's method. The smear shows small (2 to 5 μ) intracellular, oval, round or apiculate yeast like cells. They may be seen in mononuclear cells or occasionally in polymorphonuclear neutrophils.

Cultures are made in blood agar and Sabouraud's glucose agar from pus or other materials but when the material is contaminated, cultures are made on blood agar plates with 2 units of penicillin and 10 units of streptomycin per cc of the medium. Culture may also be done in Allison's copper sulphate trypticinised serum agar for primary isolation.

Biopsy is very helpful in the diagnosis of the disease. This may be obtained from the lymph node, skin, mucous surface and smears and cultures should also be taken at the same time. Histological study is specially meant for the study of intracellular parasites and cellular reactions. The lesion is essentially a granuloma in which endothelial cells are parasitised by the organism. Macrophages in the organs involved will show similar parasitisation.

Treatment Compounds of antimony, sulphonamide, penicillin, streptomycin, radium and Roentgen therapy have been used but with little success.

CHROMIOBLASTOMYCOSIS

Definition Chromoblastomycosis is a chronic infection of the skin characterised by development of verrucose cutaneous lesions with rare metastasis in the lymph node. It is caused by three species of fungi namely *Phialophora verrucosa*, *Hormodendrum pedrosoi* and *H. compactum*.

History Pedroso (1911) in Brazil first observed the characteristic black bodies in a biopsy material and isolated the organism which he failed to identify. Lane (1915) Medlar (1915) found spherical bodies in the lesion and isolated the fungus called *Phialophora verrucosa*. Pedroso and Gomes (1920) reported four cases of verrucose dermatitis (including the original case of Pedroso) caused by *P. verrucosa* but the fungus was later studied by Brumpt (1922) who renamed it *Hormodendrum pedrosoi*. Terra et al (1922) named the disease caused by this fungus chromoblastomycosis. Moore and Almeida (1935) however found one of these fungi isolated from four cases of Pedroso and Gomes to be *P. verrucosa*. The third fungus namely *Hormodendrum compactum* was identified by Carrion (1935) as the causative organism of chromoblastomycosis. Carrion thus believes that there are at least three species responsible for chromoblastomycosis.

Geographical distribution The disease has been reported from Cuba, Puerto Rico and other parts of South America, U.S.A. and stray cases have been reported from Africa, Australia, Russia, Canada, Japan, Sumatra and Java. Recently one case has been reported in India by Kakoti and Dey (1957) in a woman with metastasis in the lymph node.

Etiology Lesions are often seen on the exposed parts of the body, commonly the feet. The disease is also seen in manual labourers and wood cutters. Male adults are often the victims.

Source of infection In a review of 102 cases Weidman and Rosenthal (1941) found that the lesion developed following an injury by wood in some form. Conant and Martin (1937) found that strains of *Phialophora* isolated from wood were morphologically and serologically identical with *P. verrucosa* causing chromoblastomycosis in man.

Mode of infection The infection is often associated with a history of injury with wood and it appears that the fungus enters into the skin and subcutaneous tissue through an injury caused by wood.

Mycology Examination of the infective material (pus, tissue or lymph gland) under potassium hydroxide or sodium sulphide solution shows thick walled single double or multilocular cells 6 to 12 μ in diameter (Pl. VI c, d and g). Different species can be identified in cultures.

Culture Exudate, pus or biopsy material may be utilised for this purpose. The material is inoculated in Sabouraud glucose agar and incubated at room temperature. The cultures are kept at least for 4 weeks before they are discarded as negative.

CULTURAL CHARACTERISTICS *H. pedrosoi* and *P. verrucosa* are slow growing felt like dark brown or olive black growths whereas *H. compactum* shows a heaped up greenish or dark olive green colour (Pl. VI h). On microscopic examination of mounts in lactophenol mycelia are 2 to 3 μ in diameter and brown in colour. In *H. pedrosoi* three types of spores are seen (Fig. 17).

- (a) *Hormodendrum* type These are characterised by conidiophores bearing chained conidia which are brown, single celled and branching in character.
- (b) *Acrotheca* type In this conidiophores develop terminally or as single lateral

branches on aerial mycelia. The conidiophore is club shaped from which conidia sprout on short protuberances (c) *Phialophora* type—This is characterised by flask shaped conidiophores (phialides) containing masses of conidia. *H. compactum* is differentiated from *H. pedrosoi* by its terminal and lateral conidiophores which bear subspherical conidia in compact masses (Pl VI i). *P. verrucosa* is characterised by phialophora type of conidia but occasionally hormodendrum type of conidia are seen.

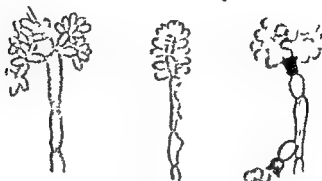


Fig. 1 Left—Hormodendrum type middle—acrothec type and right—Phialophora type of conidiophore

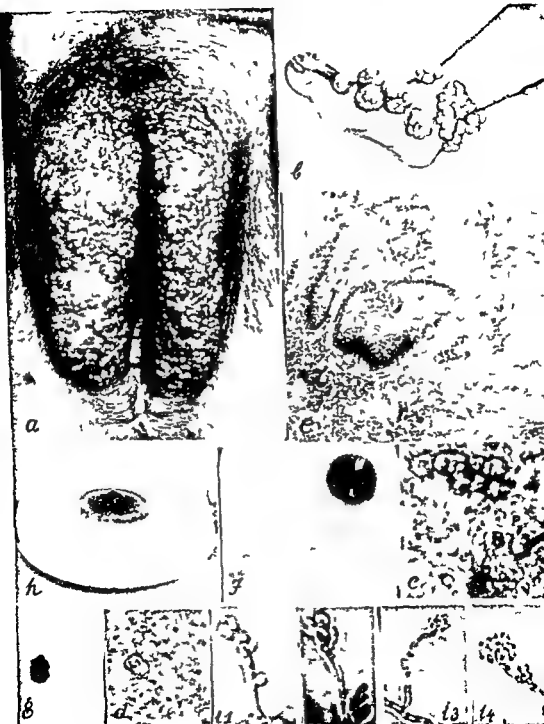
Histopathology (Pl VI e) The epidermis undergoes hyperplasia and hyperkeratosis with acanthosis and elongation of rete pegs. A granulomatous lesion is seen in the corium forming pseudotubercles formed by epithelioid cells and Langhans type of giant cells. Surrounding the granuloma are lymphocytes plasma cells eosinophils and a few neutrophils. The multilocular spores separated by septa are seen in the giant cell or in the abscess. The size of the cell is 5 to 10 μ in diameter. They appear as dark brown thick walled cells separated by septa but without any evidence of budding as in the case of blastomycosis. They reproduce in the tissue by septation (Pl VI c d and g).

Signs and symptoms The lesion is often unilateral usually on the foot or leg. It starts as a papule or pustule which gradually develops into a verrucose or cauliflower like growth. Satellite lesions develop along the lymphatics in close proximity to the primary lesion till the whole foot or the whole limb is involved (Pl VI b). Elephantiasis may result from fibrosis and lymph stasis (Pl VI a). Metastasis to the regional lymph gland is rare.

Immunology Complement fixing antibodies have been demonstrated in the serum of the patients suffering from chromoblastomycosis. But the test

Explanation of Pl VI Chromoblastomycosis (a) Verrucose condition of the leg (b) Affection of the leg and foot (c and d) Spore in tissue (KOH preparation) (e) Histopathological section showing an epithelioid abscess formation (f) Same under oil immersion showing a pair of spore (g) Spore in pair after integration of the lymph node in KOH solution (h) Culture in Sabouraud's glucose agar (i) — (j) Conidia of *H. compactum*

PLATE VI
CHROMIOBLASTOMYCOSIS



is of little diagnostic value as the fungus is easily demonstrated in the smear culture or biopsy material. Conant et al 1937 have demonstrated that the serum of the patient infected with *H. pedrosoi* fixes complement in presence of *H. pedrosoi* and *P. verrucosa* but there is no cross fixation of complement with other pathogenic fungi or plate contaminants of the genus *Hormodendrum*.

Animal inoculation. No laboratory animal has been found to be susceptible in our hands to *H. compactum*.

Laboratory diagnosis. This can only be done by finding of the fungus either in smear culture or biopsy material.

Treatment. Small isolated lesions may be excised surgically or treated by electrocoagulation. Sodium iodide is administered intravenously supplemented by potassium iodide by oral route. Sulphonamide acts in combating secondary infections and for this sulphamerazine should be tried since this has been found to be effective *in vitro*. Success has been reported in iontophoresis with copper sulphate.

CANDIDA ALBICANS (Robin) Berkhout 1923

Genus *Candida*

History. Lagenbeck (1839) demonstrated a yeast like fungus in the lesions of thrush which was named by Robin (1853) as *Oidium albicans*. The organism was called by Zopf (1866) *Monilia albicans*. Cristellari (1905) found it as the causative organism of bronchomoniliasis. The generic name *Candida* was suggested by Berkhout (1923) to include fungi which reproduced by budding and developed pseudomycelia. Since then a number of species was included in this genus. An excellent review of yeast like fungi was given by Skinner (1947). The generic name *Candida* was supported by Dodge (1935).

General characters. The genus *Candida* is characterised by yeast like fungi which reproduce by and form septate budding mycelia whereas torula reproduce exclusively by budding without formation of mycelia. It differs from *Saccharomyces* or true yeasts by presence of its mycelia which are absent in true yeast. On the other hand members of the genus *Candida* do not show ascospores seen in *Saccharomyces*. The morphological counterpart of the genus *Candida* in the class *Ascomycetes* is *Endomycopsis* which shows not only mycelia and budding but ascospores also (Fig 7).

SPECIES. There are several species of *Candida* found in nature and the common species that are commonly seen are *C. albicans*, *C. tropicalis*, *C. pseudo-tropicalis*, *C. krusei*, *C. parakrusei*, *C. stellatoidea*. Of these *C. albicans* is found often as a parasite and occasionally as a saprophyte. It is also pathogenic to rabbits. Many other species of *Candida* are mostly seen as saprophytes and are not pathogenic to rabbits. For this reason the modern trend amongst the mycologists is to accept *C. albicans* as the common pathogenic species.

Morphology. *C. albicans* is a small budding yeast like fungus 2.5 by 4 to 6 μ . In the exudate sputum and mucous membrane budding and pseudomycelial forms are seen by elongation of the cells and rarely the mycelial form. Mycelial forms are best seen in cultures showing clusters of

spores (Fig 18a) *C. albicans* in culture is differentiated from other species by the presence of chlamydospores which develop best in corn meal agar (Fig 18b). Spores are often seen in the infected skin (Fig 19)



Fig 18—(a) Blastospores in culture
(b) Chlamydospores and blastospores
in corn meal agar

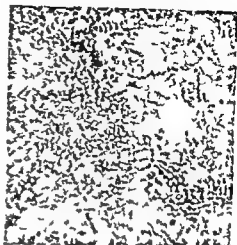


Fig 19—Spores of *C. albicans* in scales

Cultural characters. *C. albicans* grows easily in Sabouraud's glucose agar at the room or incubator temperature in about 48 hours. The colonies are yeast like, white or cream coloured and emit a peculiar yeast like odour. Giant colonies when old show honeycomb appearance in the centre and may show radial furrows (Fig 20).



Fig 20—Giant colony of
C. albicans

Biochemical reactions. It ferments glucose and maltose with production of acid and gas. It does not ferment lactose but ferments sucrose with production of acid only. Differentiation of *C. albicans* from other nonpathogenic species is shown in table V.

Animal inoculation. Intracutaneous inoculation in guinea pigs causes local abscess formation in 48 hours. Intravenous injection of 1 c.c. of a one per cent suspension of the growth in rabbits produces military abscesses in the kidney and other parts of the body and kills the animal in 4 or 5 days.

Immunity. Sera of individuals showing moniliasis often show agglutination with saline suspension of *C. albicans* but others also may show agglutination in low titres. Positive results are seen by skin tests with oidiomycin but it is of doubtful diagnostic significance as presence of the organism in the mouth and gastrointestinal tract of apparently healthy individuals may account for both agglutination and skin sensitivity tests.

MONILIASIS

Definition. Moniliasis is an infection caused by a yeast like fungus *C. albicans* affecting the mucous membrane of the mouth (thrush), intestine

TABLE 1 DIFFERENTIAL DIAGNOSIS OF SPECIES OF CANDIDA
(After Martin et al J Bact 34 99 1937)

Species	Sabouraud agar and broth	Colonial character		Glu- cose	Mal- tose	Suc- rose	Lac- tose	Patho- genicity
		Mould agar	Corn meal agar					
<i>C. alb.</i>	Typical creamy growth no surface growth	Dull gray in size of colonies	Tree-like branching mycelium with terminal chlamydo spores	AG	AG	A	—	Pathogenic
<i>C. tropicalis</i>	Not characteristic in broth film	Grayish white large colonies with mycelial fringe	Tumescence with well developed filaments to pores but no chlamydo spores	AG	AG	AG	—	Non pathogenic
<i>C. parapsilosis</i>	Not characteristic no surface growth	Small colonies not characteristic	Mycelium poorly developed chlamydo spores absent	AG	—	AG	AG	Non pathogenic
<i>C. kr.</i>	Flat dry growth with wrinkled surface film	Small dull gray colonies—not characteristic	Crossed at angles mycelia no chlamydo spores	AG	—	—	—	Non pathogenic
<i>C. parake</i>	Creamy growth no surface growth	Small brilliant white colonies	Well developed mycelia no chlamydo spores	AG or A	—	—	—	Non pathogenic
<i>C. utilis</i>	Creamy growth no surface growth	Star-like colonies with radiating arms	Mycelia with large ball-like clusters of blastospores	AG	AG	—	—	Non pathogenic

AG = Acid and gas A = Acid — = No fermentation

vagina (vaginitis) mucocutaneous areas angular cheilitis skin (dermatitis) and nails (Paronychia) and bronchopulmonary affections. It may rarely produce meningitis or other generalised infections of the body affecting the liver spleen lymph nodes etc.

Etiology The affection is seen in the tongue or buccal mucosa of infants or old people or of debilitated persons suffering from wasting diseases like tuberculosis cancer or diabetes. Lesions are also seen in people suffering from angular cheilitis due to vitamin B complex deficiency and in sprue. Lesions on

the hand and nails are seen in housewives whose hands are macerated frequently by soaking in water and also in bakers and fruit sellers

Transmission *C. albicans* is seldom isolated from natural substrates (Skinner 1947). The infection is often of endogenous origin. The organism is naturally found in the mouth or intestinal tracts of a large percentage of normal human subjects. The skin and nail infections may occur from those sources provided the moist condition is present. Infants probably contract oral infection from the birth canal. Cutaneous moniliasis of the nipple of the nursing mother is caused by the organism of the oral thrush.

Signs and symptoms *C. albicans* causes moniliasis of the nail, skin, mucous membrane, bronchopulmonary moniliasis and rarely monilids.

Paronychia (Pl VII b c and e). It is an infection of the base of the nail due to constant maceration of the tips of fingers and toes characterised by bolster like swelling of the tissue about the bases of the nail frequently seen in housewives. The affected nails show transverse ridges, thickening and distortion (Pl VII Figs c e and g).

Erosio interdigitalis blastomycetica. The fungus is apt to cause infection of the skin of the intertriginous regions namely webs of the fingers (Pl VII a and g) and toes, folds of breast, axillae, inguinal and intergluteal regions. The skin is found to be sodden and macerated in the affected areas and scraping shows a large number of the organism.

Perleche (Pl VII d f and h). It is often seen in cheilosis due to vitamin B complex deficiency. There is soddening of the angles of the mouth with signs of erosion.

Thrush. *C. albicans* causes thrush specially in marasmic children. The mucous membrane of the tongue, buccal cavity, pharynx, tonsils and gum show a scum, grayish white in colour. The patches can be removed easily with a gauze. The infection probably occurs from the birth canal of the mother at the time of birth of the child. The perianal region of the infant may be found to be infected in cases of oral thrush.

Vaginitis. Vaginitis caused by *C. albicans* is not uncommon during pregnancy. It may set up vaginal discharge as a result of inflammation caused by the fungus. It may also set up a condition of pruritus vulvae particularly in diabetic subjects.

Bronchopulmonary moniliasis. The fungus may cause a condition of bronchitis which often extends to the lungs. Cough and dyspnoea are the most distressing symptoms and haemoptysis may occur in certain cases. The sputum is mucoid and gelatinous and contains gray flakes of mycelia and detritus. Chest signs are those of bronchitis. Castellani has described three clinical types. The *mild type* is usually afebrile but in the *intermediary type* there is slight rise of temperature. Recovery may be spontaneous or the disease may progress to the *severe type* and end fatally with cough, haemoptysis, hectic rise of temperature and emaciation. At this stage it resembles tuberculosis of the lung. In the pulmonary type it may produce patchy bronchopneumonia or it may

PLATE VII

MONILIASIS



(a) Interdigital aff. — on — e osso interdigital blastomycetia (b v and) Paronychia
 (bolster like swelling) (d f and h) Moniliasis associated with (g and i)
 Deformity of the nail with successive infections in case of paronychia

involve one or more lobes with evidence of consolidation and pleural thickening. The condition of generalised moniliasis is rare in pulmonary moniliasis.

Radiologically the condition shows nonspecific type of peribronchial thickening with a hazy type of linear fibrosis.

Monilids These are sterile vesicular grouped secondary lesions with erythema and may appear on the body as a result of dissemination of the fungus in the blood. Primary focus may be seen in other parts of the body either in the skin or gastrointestinal tract.

Diagnosis It depends on the finding of the fungus from the scraping of the skin, nail, mucous surface or in the sputum or stools.

Direct examination of the scraping of the skin shows spores in large clusters and a few mycelia if any (Fig. 19); whereas direct examination of the scraping from the mucous surface under a coverslip preparation with McGuire's stain shows mycelia with budding cells stained purplish blue. **Culture** from various lesions shows almost a pure culture of *C. albicans* in Sabouraud's medium. Diagnosis of the infection of the lung is confirmed by isolation of the organisms from the sputum constantly and in large numbers. **Presence of antibodies** in the serum, namely agglutinin in high titre, precipitin or complement fixing antibodies are suggestive of infection with *C. albicans* but it is difficult to say whether there is any primary infection or not as low titre of these antibodies may be found even in intestinal conditions without any symptom.

Treatment In vulvo vaginitis, glossitis and mucocutaneous infections local application of gentian violet is advisable. But along with the local treatment administration of vitamin B-complex and adexoline in children are very good adjuvants. In erosio interdigitalis blastomycetica or paronychia application of lotio gentian violet or triple dye is useful and when continued for some months the condition heals up completely. Application of Pot. permanganate 1/4000 is useful in local lesions. In bronchopulmonary moniliasis Pot. iodide may be administered up to the dose of tolerance. Ethyl iodide insufflation has been used successfully in these cases but hypersensitive cases should be desensitised before administration of iodide. (See blastomycosis). Intravenous injections of gentian violet (5 mg. per kilogram of body weight) may be administered daily or every other day. Solutions stronger than 0.5 per cent should not be administered intravenously as there is chance of thrombosis in such a case.

TINEA VERSICOLOR AND MALASSEZIA FURFUR

Synonym Pityriasis versicolor, Tinea flava, tinea nigra, etc.

Definition The disease is characterised by scaly lesions of the face and body and associated with hypopigmentation and discolouration of the skin of the affected parts.

Etiology The disease is common in young adults of both sexes but it is more common in males than in females. It is rare in children. In the winter season the disease may remain latent but with the advent of the summer there is

a rapid spread of the disease. This can be explained owing to excessive perspiration as hyperhidrosis often predisposes to it.

Myecology. *Malassezia furfur* is the causative organism of tinea versicolor. **Direct examination.** The fungus can be demonstrated in the scales under a coverslip preparation with caustic potash but better results are obtained with McGuire's stain which stains the spores and mycelia purple (Pl VIII). When properly prepared the affected scales can be identified under the low power and under the high power characteristic morphology of *M. furfur* is seen namely (a) groups of spores with double contour (b) mycelia which are short stout sinuous often septate with blunt ends and occasionally branching.

Culture. There is a conscientious of opinion whether the organism can be cultivated but the author is of opinion that the organism can be cultivated in the same way as that of *P. ovale* (Method of Papanicolaou). From the primary growth subcultures may be obtained in glucose or glycerol agar containing sterile olive oil spread on the surface of the slope. Budding forms are frequently seen in the culture. The organism has great similarity with *P. ovale* and their difference is shown in Table VI.

TABLE VI—COMPARISON BETWEEN *M. FURFUR* AND *P. OVALE*

Particulars	<i>M. furfur</i>	<i>P. ovale</i>
Microscopical (McGuire's stain)		
Mycelia	Always present	Rare
Budding	May be present	Always present
Spores (Double contoured)	Plenty	Absent
Culture		
Growth		Difficult to grow on or in dry media
Petroff's medium		Heaped up chalky in appearance
Oil media		Heaped up glaucous growth

Signs and symptoms. The disease starts as tiny hypopigmented scaly macules on the face chest neck back or abdomen. The macules coalesce and form hypopigmented areas which exhibit a wide diversity of shape and size. On close observation the macules often gyrate due to coalescence. They are seen to be covered with shiny scales and show yellowish brown or dark colour in contrast to the healthy skin. According to the colour they have been variously called tinea flava tinea alba or tinea nigra. The condition is non-inflammatory and does not produce any subjective symptoms except a little irritation or discomfort specially when exposed to the sun. The disease causes mottled disfigurement.

Plate VIII

MASSERIA FULVA



scales of *Pinea vericolor* (McGuire's stain) showing spores in cluster and mycelia scattered irregularly budding forms are also seen (Some of the cells are nucleated (Parakeratosis)

To be Aug 11

Plate IX
TINEA VERSICOLOR

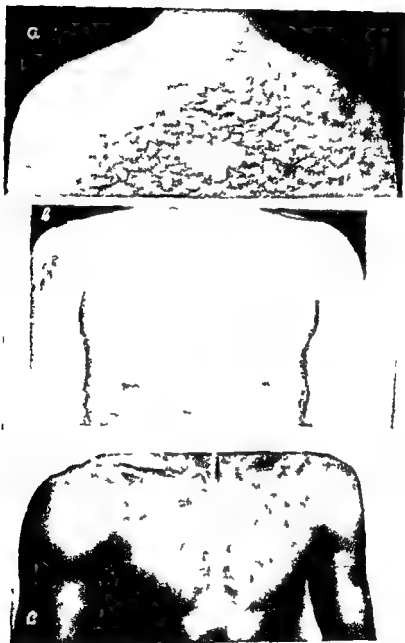


Fig: (a)—back (b) and (c)—upper part of the chest

of the neck face and trunk (Pl IX a b c) and interests the patient mainly from the aesthetic point of view. The affected parts show fluorescence under the Wood's light.

Diagnosis. This can be done directly by examination of the scale scraped from the lesion. When McGuire's method is used the morphology of the stained fungus can be very well studied, namely groups of spores and mycelia. Indirectly the condition can be diagnosed by demonstration of fluorescence under the ultraviolet light passed through Wood's filter and the colour may vary from a light to dark brown according to the degree of discolouration.

Differential diagnosis. Hypopigmented macules are often confused with (1) Dermal leishmanoid (2) Neuromacular leprosy.

(1) **Dermal leishmanoid.** Hypopigmented macules of dermal leishmanoid are erythematous rather than scaly. Scrapings do not show presence of *M. furfur*. Characteristic feature of dermal leishmanoid is that the granuloma more often affects the mask area of the face with an erythematous flush. In well advanced cases small nodules may be present specially on the chin. In case of dermal leishmanoid the patient always gives a history of kala-azar. Smears from the erythematous hypopigmented lesions do not usually show *Leishmania donovani*.

(2) **Neuromacular leprosy.*** In this condition there is always partial loss of pigment as in a case of tinea versicolor. The patch is hypopigmented and anaesthetic but rarely there is hyperaesthesia or parasthesia. Deep analgesia is often present. The scraping from the lesion does not show any fungus.

Treatment. Sulphur in any form will effect a cure (for details see seborrhoea). To avoid recurrence personal cleanliness is essential and under-wears or garments coming in direct contact with the skin should be changed and boiled daily to avoid reinfection. Pulv. Sulphur et camphor is very useful in cases of profuse perspiration.

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CHAPTER VI

SUBORDER BLASTO ARTHROSPORINEAE

Order Thallosporales Vuillemin 1910

GEOTRICHOSIS

Definition It is a condition caused by *Geotrichum candidum* affecting the skin mucous membrane gastrointestinal tract infection of the lung simulates chronic pulmonary tuberculosis

History *G. candidum* was isolated from leaf mould by Link (1809) This was often called *Oidium* or *Oospora lactis* The term *Oidium* means the imperfect stage of certain fungi of the class Ascomycetes the powdery mildews parasitic on plants However to give effect to priority the species is called *G. candidum* and this term has already become popular in the field of mycology

Habitat This occurs in the soil as a saprophyte and has also been isolated from stools and lesions in the human body

Mycology *G. candidum* appears in the form of arthrospores which are characteristically rectangular 4 to 6 μ by 8 to 12 μ in size Thick walled ovoid or round cells may form a chain of arthrospores Rounded forms may be mistaken for Blastomycetes but presence of arthrospores in chains is diagnostic of *Geotrichum*

Culture The organism grows quickly in Sabouraud's glucose agar either at room temperature or at 37 C The growth is membranaceous and in about 7 to 10 days colonies form They are flat white to cream coloured soft to the touch of the loop like yeast colonies and can be picked up easily In liquid media it forms a surface membrane

Microscopically arthrospores are round or rectangular in shape They give out germ tubes but do not reproduce by typical budding

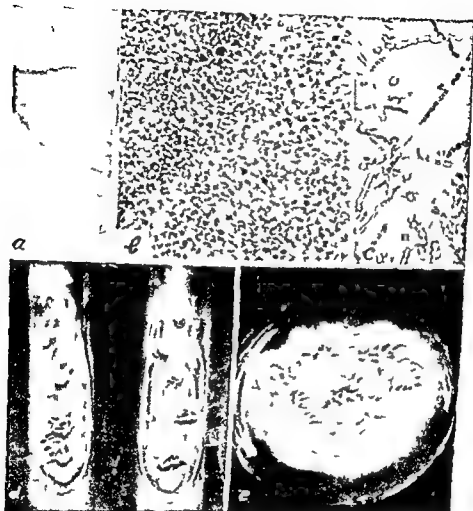
Biochemical reaction Most of the strains liquefy gelatin and produce acid in certain sugars Some strains coagulate milk

Symptoms It may produce a condition of bronchitis with persistent cough The sputum is often grayish but it may be blood stained occasionally X ray examination shows peribronchial thickening The general health of the patient is however not affected Infection of the lung may simulate chronic tuberculosis Oral geotrichosis resembles thrush caused by *Candida albicans*

Treatment Potassium iodide by mouth and sodium iodide intravenously have been successfully used in cases of pulmonary geotrichosis

PLATE X

TRICHOSPORUM BEIGELII



(a) Nodules of the hair (low power) (b) Same under higher magnification (c) Hanging drop culture showing arthrospores in chains (d) Growth of *T. beigelii* on Sabouraud's medium—three weeks old (e) Giant colony six weeks old

PIEDRA

Synonyms Black piedra White piedra *Tinea nodosa* Beigel's disease etc

Definition Piedra is a fungus infection of the hair of the scalp moustache or other hairs of the body caused by *Trichosporon beigelii* producing white, nodules easily detachable from the hair and *Piedraia hortai* producing black piedra, forming hard nodules firmly adherent to the hair. The condition is contagious.

History The disease was first described by Beigel (1865) in Germany forming nodules on the hair of wigs. Behrend (1890) described *Trichosporon ovoides* recovered from the hair of the scalp in Germany. Vuillemin (1902) found light coloured nodules on the moustache and called it *T. beigelii*. Black piedra was originally described by Dessene (1878). Horta (1911) studied black piedra of the hair in Brazil and Fonseca and Leão (1928) called it *Piedraia hortai*. According to the work of McKinnon (1942) black piedra in Brazil is due to *P. hortai*, white piedra in South America is due to *T. beigelii*, other species namely *T. giganteum* Unna 1895 *T. ovoides* Behrend 1890 *T. glycophila* Dubois 1910 etc fall synonymous with *T. beigelii* (Rabenhorst) Vuillemin 1902.

Geographical distribution White Piedra occurs in South America central Europe England and Japan. Dey and Kakoti (1954) reported a case of white Piedra in Assam. Black piedra has been reported in Columbia and other countries of South America and from East Indies Java and Kochin China.

Etiology *P. hortai* infections are seen in epidemic form more often in the moustache of man than in the long hair of woman.

Mycology Direct examination *T. beigelii* forms a mass of gelatinous mycelial spores composed of round or oval cells. In a coverslip preparation with sodium sulphide the arthrospores are found to from 3 to 10 μ in diameter. The fungus reproduces by arthrospores and also by budding and both the elements are held together in a gelatinous substance (Pl X a b). In a coverslip preparation with sodium sulphide solution nodosities of the infected hair in case *P. hortai* contain round or polygonal cells. They form dark hard adherent mass formed by broad closely septate hyphae branching dichotomously. Numerous oval asci 30 by 50 μ are seen containing eight curved or fusiform ascospores 10 by 30 μ having simple terminal cilia like appendages.

Culture On Sabouraud's medium *T. beigelii* grows faster and forms a soft brittle cream coloured wrinkled colonies (Pl X d e). They show thick double walled arthrospores forming chains (Pl X c). On Sabouraud's glucose agar *P. hortai* forms a slow growing greenish black glabrous growth hard to touch nodular or wrinkled in appearance.

Pathogenicity *T. beigelii* causes sporadic infection of the long hair of the scalp and is much less contagious than *P. hortai* whereas *P. hortai* affects the coarser hair of the beard and moustache rather than the scalp hair.

Treatment The hair should be cropped close to the crown or shaved and lotio hydrarg. perchloride (1:2000) or Ung. Hydrarg. ammon. (3 per cent) should be applied on the scalp. Lotio resorcin is equally efficacious in this condition.

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CHAPTER VII

SUBORDER ARTHROSPORINEAE

Definition These are Thallosporites which reproduce by arthrospores

Classification Genus *Madurella* Brumpt 1905 producing black mycetoma
Genus *Indiella* Brumpt 1906 producing white mycetoma

GENUS MADURELLA Brumpt 1905 emendata Pinoy 1912

Definition and general characters These are Arthrosporineae characterised by sterile septate hyphae which reproduce by arthrospores often secreting a black pigment. These are generally found in black mycetoma and grow well at 37°C. The type species is *M. mycetomi* Laveran 1902

MADURELLA MYCETOMI Laveran 1902

Geographical distribution Carter's black grain mycetoma is found in India, Africa, Somaliland, Senegal, Madagascar, Algeria; one case has been reported from Italy.

Morphology and cultural characters The filaments are 1 to 4 μ in diameter and the arthrospores which are formed by grey hyphae are 3 to 10 μ . Each of the arthrospores divides to form two spores which are of the same diameter. The membrane becomes yellowish with age. Chlamydospores may be 10-12 μ in diameter, intercalary or terminal at the end of filaments like fusic nris (Fig 21).

Culture They form black sterile sclerotes 0.5 to 1 mm often formed in the depth of the medium.

Pathogenicity The organism causes Carter's black mycetoma in man invading the skin, muscles, bones and connective tissues. They form granulomas with grey or black granules 1 to 2 mm in diameter. The granules are hard, brittle, round and warty, often resembling the sclerotes in culture media.

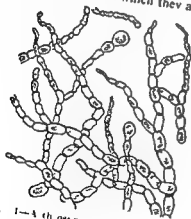


Fig. 1—A chain of arthrospores of *M. mycetomi*

MADURELLA TOZEURI Nicolle and Pinoy 1904

In the lesion they show mycelial elements with yellowish membrane 1 to 4 μ in diameter. They often give a speckled appearance in smear media but rarely with any sclerotic formation.

Pathogenicity They produce mycetoma in man with amorphous black grains. The granules are not more than 1 mm and resemble those of Carter's mycetoma. The granuloma is often associated with giant cell formation. The pigment is formed by tyrosinase.

GENUS *INDIELLA* Brumpt 1906

Definition and general characters These are Arthrosporineae with septate branching hyphae without any pigment forming white or yellowish small granules. Mycelial threads are 1 to 8 or 10 μ in diameter. Masses of mycelia form sclerotia like bodies or grains containing chlamydospores in the tissues of men and animals. Mycelia are known only in the parasitic life and has not yet been cultivated. Filaments are 3 to 5 μ in diameter. Septate ramified septa are seen at a distance of 5 to 10 μ . In grains, chlamydospores are seen most

often terminal and the sizes vary from 5 to 12 μ in diameter. Sclerotes are small about one fifth of a mm. Type species *Indiella mansonii* Brumpt 1906.



Fig. - Sclerotia of *I. reynoldsii* in vermiform masses

Classification On the basis of sclerotia *Indiella* are classified as follows —

- 1 Sclerotia hard and bean shaped—*I. mansonii* causing Brumpt's white mycetoma in India and China
- 2 Sclerotia soft and coiled in vermiform masses—*I. reynoldsii* causing mycetoma in France and Greece (Fig. 22)

A third species has been described in Brazil—*I. Brumpti* Pirajá da Silva 1922



CHAPTER VIII

ORDER HEMISPORALES Vuillemin 1910

These are Hyphales (Hyphomycetes) which reproduce by hemisporae. Hyphae are thin but more than 1μ in diameter. Conidiophores are septate and branched. Each hemispore consists of a branch terminating in an ampuliform protoconidium preceded by an annular constriction (Fig 23 b). Protoconidium divides partially or completely into several spore like segments deuterconidia (Fig 23 c). Occasionally however it elongates forming a new conidiophore. After rupture of the tubal wall conidia remain adherent for a short period to the tubal membrane and are then set free. The conidia are rough subspherical, oval or barrel shaped and thick walled.

HEMISPORA STELLATA Vuillemin 1906

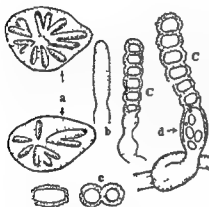


Fig 23—(a) Growth of *H. stellata* on Sabouraud's medium (b) Protconidia (c) Deuterconidia (d) Formation of deuterconidia

H. stellata produces indolent, gummatous ulcers like those of sporotrichosis. Cold abscesses or osseous lesions are sometimes produced as in tuberculosis or in tertiary syphilis. It is pathogenic to man and was first found by Gougerot and Caravan (1909).

Culture The culture shows white stellar discs covered with conidiophores. Deuterconidia are subspherical or barrel shaped 2.6 to 3.5μ with dark coloured granular membrane (Fig 23 c). Culture grows well on sugar media at room temperature.

Treatment Treatment with potassium iodide gives good results.

CHAPTER IX

ORDER CONIDIOSPORALES

They reproduce by conidia which may be microconidia (aleuriospores) or true conidia borne on sterigma

SUBORDER ALFURIOSPORINFAE

Definition : Organisms of this group reproduce chiefly by aleuriospores or microconidia. Many of the species show formation of fursiform macroconidia. Dermatophytes and the genera *Glenospora* and *Acladium* fall into this group

TABLE III DERMATOPHYTES OR RINGWORM FUNGI

Genus	Species
1. <i>Microsporum</i>	<i>M. audouinii</i> <i>M. canis</i> <i>M. gypsum</i>
<i>Trichophyton</i>	
<i>Stafurii</i> group	<i>T. parvum</i> (<i>T. debile</i>)
<i>Ceratiform group</i> (<i>Trichothrix</i>)	<i>T. trichothrix</i> <i>T. sphaerum</i> <i>T. abnormale</i>
<i>Cylindrus</i> group (<i>Trichothrix</i> & all spore)	<i>T. mentagrophytes</i>
<i>Rhizoglyphus</i> group (<i>Trichothrix</i> large spore)	<i>R. commune</i>
<i>Paecilomyces</i> group	<i>T. schoenanthii</i> (all varieties) <i>T. incarnatum</i> <i>T. lilacinum</i> (<i>Trichothrix</i>) <i>T. dermatophylus</i> (<i>Trichothrix</i> large spore)
2. <i>Epidermophyton</i>	<i>E. floccosum</i>

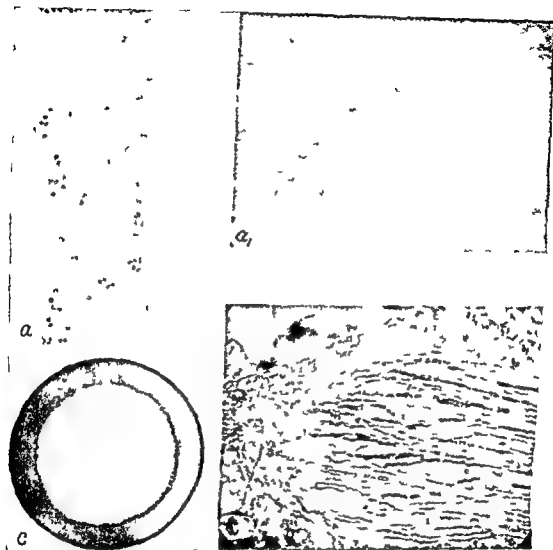
Genus *Microsporum* Gruby 1843

General characters Ringworm fungi of this group cause tinea capitis in children and the glabrous skin may be secondarily affected. The animal species may cause kerion. The root of the infected hair is covered with a sheath of spores. Microscopic examination of the infected hair shows mosaic distribution of small spores 2 to 3 μ in diameter. On microculture fusiform pointed macroconidia are seen at the end of fertile hyphae. The microconidia are of the type of *Acladium* along the side of the hyphae. Three important microspora which cause infection of the hair are

- (1) *M. audouinii* (2) *M. canis* (3) *M. gypsum*

PLATE XI

MICROSPORUM AUDOUINI



(a and a¹) Infected hair showing mosaic arrangement of small spores. Adamson's fringe at the bulb of the hair. (c) Giant colony of *M. audouini*.

MICROSPORUM AUDOUINI Gruby 1843

Synonyms *Trichophyton decicans* *Sabouraudites audouini* *Closteriosporia audouini* *M. umbonatum* *M. veheticum* *M. tardum* *M. tomentosum* *M. depauperatum* *M. villosum*

They cause scaly ringworm of the scalp in children (not seen in Indians)

Direct examination The fungus forms a mosaic sheath around the stump of the hair (Pl XI a a₁) There is no tendency to chain formation along the long axis of the hair as in the case of trichophyta the spores are round or oval small and 3 to 5 μ in diameter Mycelia may be seen in infection of the glabrous skin

Cultural characters In Sabouraud's agar on the 3rd day after inoculation the primary culture appears as a feathery fluff and shows greyish white growth with scanty aerial mycelia With the growth of the colony radial furrows appear with a central elevation or an umbo characteristic of the growth and typically seen on Sabouraud's maltose agar (Pl XI c) Pleomorphism is not common

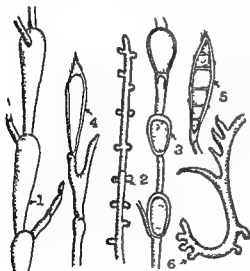


Fig. 24—1 Mycelium at a joint 2 Microconidium 3 Chlamydospore 4 Macroconidium without septum 5 Septate macroconidium with 11 segments 6 Hyphae pectinate

Microculture Fusiform macroconidia are occasionally seen microconidia are of accladium type and not in groups as in trichophyta Mycelial racquets chlamydospores and hyphae pectinate are frequently observed in Sabouraud's media (Fig 24—1 to 6) and natural media like wheat or barley

Wood's light The infected hair stumps show fluorescence as bright clear green areas Usually all the hairs in a lesion are seen to be involved In 10 days old culture it appears dull clear and mouse grey throughout

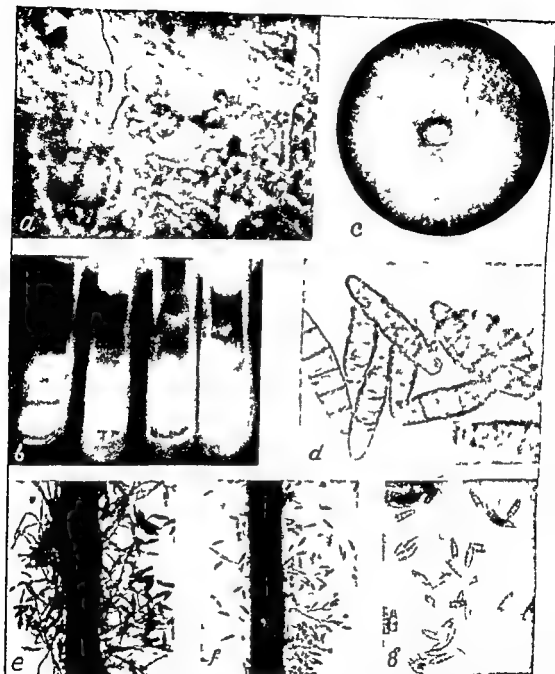
Animal inoculation Animals are resistant to infection after inoculation with this fungus

MICROSPORUM CANIS Bodin 1902

Synonyms *M. lanosum* *M. equinum* *M. felineum* *M. caninum* *M. pseudo lanosum* *Sabouraudites lanosum* *Sabouraudites lanatus* *Closteriosporia lanosa* *M. stillianus* *M. aurantiacum* *M. simiae* *M. obesum* etc

This often causes microsporiasis with kerion formation It has been recorded from Europe and America but not from India

PLATE XIII
MICROSPORUM GYPSEUM
(From favus in a rabbit)



(a) Mould in the favus at high power (x610). (b) Growth in Sabouraud maltose agar — in culture and subcultures. (c) Giant colony on Sabouraud maltose agar. (d) Macroconidia at high power (x810). (e) Hairs found naturally infected the owl housing. (f) Fungus growing from the infected hair at low power (x100). (g) Macroconidia at low power (x100).

arrangement. The infected hair shows a sheath of spores with rosaries. When the infective stage is complete the microscopic appearance of the hair is indistinguishable from that of other microspora. Lesions of the smooth skin show short chains of spores in the scale. Favic scutula show mycelia and spores. (Pl XIII r)

Cultural characters. In Sabouraud's agar the colony grows fast with brownish white powdery aerial mycelia often giving the appearance of suede. The cinnamon brown colour of the colony is characteristic. A central umbo almost invariably appears about 2 weeks after subculture. The margin of the colony is often fringed (Pl XIII c). Furrows are absent but there may be concentric rings in some cases.

Pleomorphism is characterised by growth of white sterile mycelia.

Microculture. Numerous macroconidia are produced. They are fusiform in nature 8 to 12 by 15 to 50 μ . They are pluriseptate and often ellipsoid with thin walls but tuberculated when old (Fig 26 and Pl XIII d e f g). Mycelial racquets chlamydospores

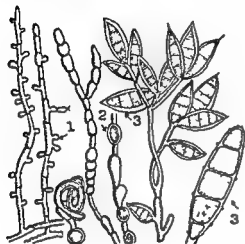


Fig 26—1 *gyp* m μ l Microconidia 2 Chlamydospores 3 Septate macroconidia tuberculated when old

and microconidia are often seen (Fig 26)

Wood's light. The infected hairs fluoresce light green as in other microspora. The growth is dull and clear cinnamon brown in colour throughout.

Animal inoculation. Young laboratory animals can be easily infected by *M. gypseum*.

Genus *Trichophyton* Malmsten 1945

General characters. They attack the hair skin and nails. The genus *trichophyton* is characterised by a large number of microconidia which are single celled thin walled hyaline round or clavate 2 by 4 μ borne en grappe or singly from the side of a hypha (thyse). The macroconidia are less numerous cylindrical rather than fusiform. In the faviform trichophyta microconidia are rudimentary and macroconidia are absent but intercalary and terminal chlamydospores are common.

Macroscopically the cultures on Sabouraud's agar are often granular as *T. mentagrophytes* but they become downy when old. In other cases they are faviform i.e. glabrous smooth and waxy. Pigment varies from species to species e.g. white pink purple violet orange yellow or brown.

CLASSIFICATION. Different groups of this genus are formed based on the gross macroscopic appearance of cultures. They are *T. purpureum*, *T. crateriforme*, *T. mentagrophytes*, *T. rosaceum* and faviform trichophyta.

produced along the mycelium or en grappe Chlamydospores, mycelial racquets and nodular organs are also present. Spirals are characteristic

Wood's light In young culture the colour is bright and light blue throughout the colony but in the fluffy growth it is seen only along the periphery. In older colonies a light blue border is characteristic

Animal inoculation It is occasionally successful

CRATERIFORM GROWTH

TRICHOPHYTON TONSURANS Malmsten 1845

Synonyms *T. crateriforme* Sabouraud 1902 *T. effractum* Sabouraud 1910 *T. fumatum* Sabouraud 1916 *T. regulare* Sabouraud 1910 *T. exsiccatum* Sabouraud 1910 *T. polygonum* Sabouraud 1910 *T. umbilicatum* Sabouraud 1910 etc

The organism causes endothrix trichophytosis or black-dot ringworm of the scalp and this is generally seen to infect children in France and other parts of Europe. But seven cases were recorded by Dey and Miplestone (1941) in an epidemic in a European school in Calcutta. The lesions on the scalp are small patches usually multiple without any inflammatory changes. The hairs under the scales appear like letters S or Z or twisted spirally. It might

infect the glabrous skin as an extension from the scalp; nails may be affected secondary to scalp infection. In smooth skin the lesions are circinate non-inflammatory with scale formation (*Tinea circinata*)

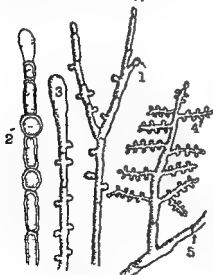


Fig 25 *T. tonsurans* (1) Microconidium (2) Chlamydospore (3) Terminal club (4) Microconidium on compound hair

Direct examination In the hair spores are large seen in chains along the hair shaft (Pl XV a b). Mycelial filaments are 4 to 5 μ in diameter and old mycelia show spores with square-cut ends. The spores are differentiated from those of other endothrix trichophytes e.g. *T. accuminatum* or *T. violaceum* which are oval in shape.

Cultural characters The growth is slow and shows a white or cream coloured velvety surface with a central crater which develops with the age of the culture. The centre becomes folded

with irregular radiating furrows (Pl XV c d e). The colour in the central portion of the growth is gradually transformed into yellow with age. Pleomorphism is rare.

Microculture Macroconidia are rare and when present they are rudimentary club shaped and poorly formed. Microconidia are numerous held either

laterally or en grappe often with a compound branching arrangement (Pl. XV, g h). They are either sessile or borne on short sterigma. Chlamydospores are abundant (Fig. 28).

Wood's light. With filtered ultraviolet rays the infected hair appears dull white in colour but in culture the growth shows a dark olive colour throughout.

Animal inoculation. Spontaneous healing is observed when guinea-pigs are inoculated with the fungus.

TRICHOPHYTON SULPHUREUM Sabouraud 1910

This fungus is common in England and is very similar to *T. tonsurans*. In children it produces lesions of the scalp and glabrous skin.

Direct examination. Spores are large 4 to 5 μ in diameter and seen in chains inside the hair shaft. Scraping from the lesion of the smooth skin shows mycelial filaments.

Cultural characters. The growth is fairly rapid. The colony is velvety at first with a central nodule showing a delicate primrose colour. With the age of the culture the growth becomes folded with a small crater in the centre and the colour soon changes to sulphur yellow. On subculture the growth is rather fluffy covered with a lighter yellow colour and the central pigment is often lost.

Microculture. Microconidia are numerous and are seen laterally and en grappe. Macroconidia if any are rudimentary or lacking. Chlamydospores are often seen.

Wood's light. Infected hairs show a pale grey colour.

Animal inoculation. Results are irregular.

TRICHOPHYTON SABOURAUDI Blanchard 1896

Synonyms. *T. acuminatum* Bodin 1902. *T. pilosum* Sabouraud 1910.

It is responsible for endothrix trichophytosis in France and essentially resembles *T. tonsurans* in its cultural and morphological characters.

GYPSEUM GROUP

TRYCHOPHYTON MENTAGROPHYTES (Robin) Blanchard 1896

Synonyms. *Microsporium mentagrophytes* Robin 1856. *Achorion quinquatum* Blanchard 1896. *Trichophyton gypseum* Bodin 1902. *T. equinum* Geddolet 1902. *T. granulosum* Sabouraud 1909. *T. radiolatum* Sabouraud 1910. *T. asteroides* Sabouraud 1910. *T. lacticolor* Sabouraud 1910. *T. niveum* Sabouraud 1910. *T. radians* Sabouraud 1910. *T. denticulatum* Sabouraud 1910. *T. interdigitalis* Priestly 1917. *T. C.* Hodges 1921. *T. kaufmanni* Wolf 1922. *T. pedis* Ota 1922 etc.

This group consists of at least three different types namely (1) *T. gypseum* or granular type (2) *T. interdigitalis* or the white fluffy type and (3) *T. niveum* or the compact powdery growth. Immediately after isolation from

the hair or rarely from the skin the granular type of growth is very characteristic. On repeated subcultures of the granular type or very often after isolation from the skin the fluffy growth is seen giving the appearance of *T. interdigitale* and this is rarely reversible to the original type. The gypsum type is the animal adapted strain and produces the maximum inflammatory reaction of the hair follicles. *T. interdigitale* on the other hand is a human adapted strain but it has also a tendency to mild inflammatory reaction in cases of tinea pedis particularly on the insteps of the foot. In the latter condition the inflammatory reaction is very characteristic. *T. niveum* is a degenerated form of *T. interdigitale* and a culture of *T. interdigitale* when old gives a similar growth character. In all these types pigmentation in the depth of the medium is present and characteristically dark rose tan.

Direct examination. Hair shows the character of an ectothrix trichophytosis and the fungi are seen outside the hair. The spores are small (Pl. XVI a) like those of microspora. In vesicles or macerated skin *T. interdigitale* appears as short mycelia often with shreds with little branching (Fig. 29).

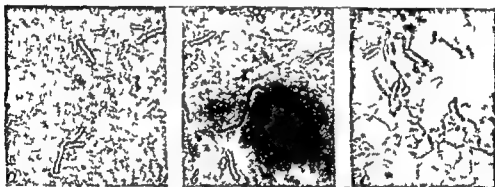


Fig. 3—(a and b) *T. interdigitale* with strands of mycelia in the skin of human foot. (c) Mouldy fungus in the blister skin without a true mycelium, this is due to deposit of cholesterol.

Cultural characters. In Sabouraud's agar the growth of *T. gypsum* is characterised by coarsely powdery surface. The growth is very rapid and the whole surface of the Frienmeyer's flask is covered in 4 to 6 weeks by the growth. In certain cases fluffy growth may appear in certain sectors of the growth during this period. The colour is usually dark rose tan. In the gypsum growth a few concentric rings may appear on the surface. The margin of the growth is often fringed (Pl. XVI b).

T. interdigitale is characterised by a white fluffy growth from the beginning and after a variable period of subculture it passes to the *niveum* type when the growth is fully degenerated and pleomorphic. In such a case the whole surface of the medium is covered by a snow white friable growth. The growth which is powdery comes out in flakes when touched with a loop.

Microculture. *T. gypsum* shows numerous macroconidia with either 3 or 4 septa. Microconidia are almost always en grappe and also

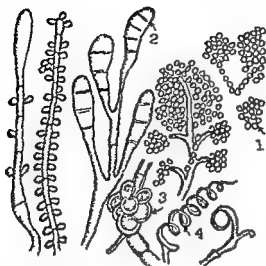


Fig 30 *Trichophyton gypsum* (1) Microconidia en grappe (2) Macroconidia with a few apical spines (3) Nodular organ (4) Spirals

Wood's light In ectothrix trichophytosis of the scalp or beard areas fluorescence is absent the skin or nails also do not show any fluorescence. The granular type of growth is typically bright and shows concentric bands of colour. It is bluish violet in the centre and fawn colour at the periphery. *T. niveum* shows a uniform yellow tone.

Animal inoculation The granular type of growth (*T. gypsum*) can be inoculated successfully into guinea pigs (Pl. XVI f), cats, rabbits but the fluffy type (*T. interdigitale*) does not usually show this pathogenicity for these animals.

ROSACEUM GROUP

TRICHOPHYTON ROSACEUM Sabouraud 1910

Synonyms *Epidermophyton gallinae* Megnin 1881, *T. gallinae* (Megnin 1881) Blanchard 1896, *T. roseum* Bodin 1902, *T. megnini* Blanchard 1896, *T. vinosum* Sabouraud 1910, *Achorion gallinae* Sabouraud 1910.

This group differs slightly from the rubrum group and contains a single species with various synonyms. In man it causes *tinea barbae* or *tinea capitis* without marked inflammation, *tinea corporis* and ringworm of nails. It causes favus in chicken.

Direct examination In the hair they show ectothrix trichophytosis. The spores are $4\ \mu$ or larger in diameter and are arranged in chains outside the hair.

Culture On Sabouraud's medium the growth is characteristically cottony or velvety. At first the colony is white but later it becomes pale rose or delicate pink and the reverse of the agar is currant violet or raspberry rose. Some strains show cerebriform growth with surface cracks.

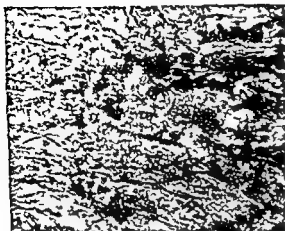
Microculture The organism occasionally shows macroconidia. Microconidia are often borne laterally and rarely en grappe. Mycelial racquets and chlamydospores may be observed.

borne laterally. Microconidia are characteristically round in this case. Spirals are typical. Chlamydospores, mycelial racquets, pectinate hyphae and nodular organs are often seen (Fig. 30).

In *T. interdigitale*, round microconidia are often seen in large clusters. Macroconidia and spirals are usually seen immediately after isolation but they gradually disappear in subsequent subcultures. Chlamydospores, mycelial racquets, nodular organs are usually present.

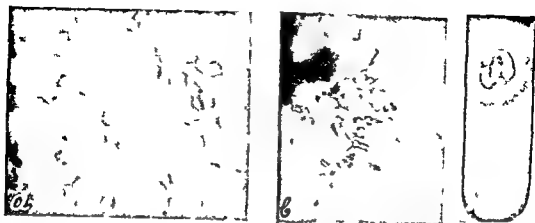
In *T. niveum* fragmented mycelia and microconidia are only seen.

PLATE XVI
TRICHOPHYTON MENTAGROPHYTES



(a) F totth x trichophyton of t g th la (gyp um) (b) R product vo end o gan nam ly ic ocon d m roconid a n l sp rals
(d) Macroconidia (e) Macroconidia anisptc lma o on da (f) Su c sf l inoculation
into a guinea pig

PLATE XVII
ACHORION SCHOENLEINI



(a) Mycelium and spores in seutula (b) Chlamydospores in microculture (c) Culture—6 weeks old

PLATE XVIII
ACHORION ACTOVI



(a) Seutula showing mycelium and spores (b) Colony (c) Colony (d) Colony
(e) Funnel-shaped microculture

FAVIFORM GROUP

TRICHOPHYTON SCHOENLEINI (Lebert) Langeron and Milochevitch 1930

Synonym *Achorion schoenleini* Remak 1845 *Oidium schoenleini* Lebert 1845

Trichophyton schoenleini causes favus of the scalp, smooth skin and nails in Europe. It is characterised by formation of scutula on the scalp with deposit of mud plaster like crust. When crusts are removed a raw surface is exposed. On healing scars are formed resulting in alopecia in patches. On the smooth skin vesicular or ringworm lesions form followed by accumulation of favic material consisting of scales and fungus mixed with secretion of a fatty nature. When the nails are affected they are characterised by hypertrophy with heaped up favic material.

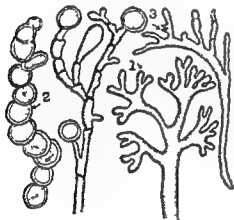


Fig 31—(1) Fungus chandeliers (2) Intercalary chlamydo spores (3) Hyphae in tissue

Direct examination Favic scutula show mycelia with masses of branching hyphae and often chlamydo spores. Hair shows endothrix infection with spores or fragments of mycelia in the hair which often contain air spaces attached to its margin.

Culture Characteristic growth appears in about 3 weeks. This shows a heaped up convex glabrous faviform cream coloured growth having a cerebiform or honeycomb appearance (Pl XVII). Pleomorphism is uncommon.

Microculture Terminal and intercalary chlamydo spores are common and numerous. The growing end of the mycelium may show favic chandeliers (Fig 31). Aleuriospores are absent in

Sabouraud's agar mycelial racquets are present. Aleuriospores have been found by Langeron and Milochevitch in natural media. This could not be confirmed by the author as it is difficult to grow the organism in these media.

Wood's light Infected hairs show greenish fluorescence. The culture gives a dull olive grey appearance throughout.

Animal inoculation All the common laboratory animals may be infected.

TRICHOPHYTON (ACHORION) ACTONI Dey and Maplestone 1936

Dey and Maplestone (1936) observed a favic trichophyton having a cultural and morphological character similar to those of *T. schoenleini* but the terminal chlamydo spores are very often funnel shaped instead of being oval or round (Pl XVIII). It is responsible for causing favus in Rajasthan and Kashmir in India.

TRICHOPHYTON VIOLACEUM Bodin 1902

Synonyms *T. album* Sabouraud 1909 *T. glabrum* Sabouraud 1909
Achorion violaceum Bloch 1911

The organism is responsible for causing *endothrix trichophytosis* of the hair characterised by black dot ringworm of the scalp but the infection may spread to the glabrous skin of the body nail or beard region

Direct examination (Plate XIX a b c d) The stump of the hair when examined under sodium sulphide solution shows mycelia containing spores in rows and often giving a beaded appearance. The spores vary from 3 to 5 μ and are larger than those of *microspora*. Mycelia are limited to the interior of the hair and not outside it (*endothrix*).

Cultural characters (Pl XIX Figs 1—4) The organism grows slowly like other faviform trichophyta. The rate of growth is about 1.5 to 2 cm in 6 weeks. The growth is faviform and shows a glabrous surface with convolutions on the surface and radial groove marked near the periphery. The primary culture gives a deep violet appearance but the violet pigment fades on repeated subculture till there may be a central pigmented portion but the surrounding portion becomes colourless and glabrous (Pl XIX Fig 4). Pleomorphism is rare.

Microculture (Fig 32) The hanging drop culture does not show any typical microconidia formation. Mycelia show septa with formation of numerous arthrospores and chlamydospores which may be intercalary or terminal like favic nails. Mycelia show irregular branching and rackets are often seen but aleuriospores are either rudimentary or absent.

Wood's light Fluorescence is not seen as in the case of *T. violaceum* infection.

Animal inoculation Animal inoculation on the skin of guinea pigs and rabbits were found to be doubtful but the inoculation in monkey was successful 10 days after inoculation and in one of the

two monkeys inoculated by the author the lesion was faviform in character (Pl XIX Fig c).

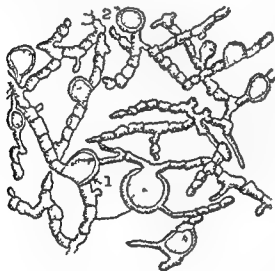


Fig 3 — *T. violaceum* Arthrospores are plenty with (1) Intercalary and (2) Terminal chlamydospores

TRICHOPHYTON CONCENTRICUM Blanchard 1896

Synonyms *Endodermophyton concentricum* Castellani 1910 *Trichophyton Castellani* (Perry) Castellani 1908 *Trichophyton mansonii* Castellani 1905 *T. indicum* Castellani 1911 *E. tropicalis* Castellani 1919 *E. roquettei* Fonseca 1925

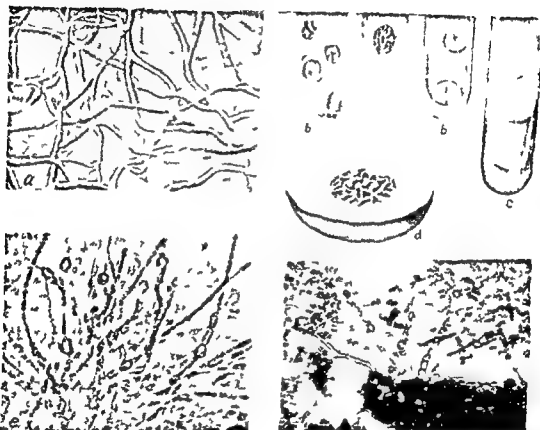
Direct examination Scales show septate intersecting mycelia (Pl XX a) easily stained with McGuire's stain or seen under a coverslip preparation with sodium sulphide.

PLATE XIX
TRICHOPHYTON TOLACEUM



(a) Interior of interior of the hair (b) Laval on a monkey
(c) Isolation in Sabouraud's medium from (1) hair (2) pus in the hair follicle (3) Subculture
(4) Loss of pigment by repeated subculture in the same medium

TRICHOPHYTON CONCENTRICUM



(a) Seal of tissue and meat showing mycelia. (b) Trunary culture from a slo. (c) Sub culture. (d) Giant colonies. (e) Micro culture showing ellamido for - (f) out of the fungus rhingine for preparation from a slo. (high power 43x).

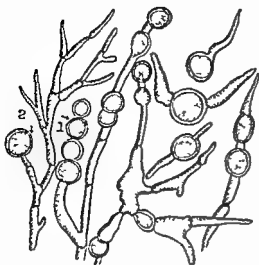


Fig 33—*Trichophyton* (1) Intercalary chlamydospores (2) Terminal chlamydospores

Cultural characters : Primary culture in solid medium is difficult owing to the fact that the redundant portion of the horny layer of the epidermis does not give any growth. Fresh material adhering to the skin should be utilised rejecting the redundant part sticking out.

For culture treat the scales in alcohol for 5 minutes and inoculate tiny bits of infected material in glucose broth, Sabouraud's glucose agar and Czapek's synthetic agar. In glucose broth the growth appears on the 3rd or 4th day and forms as a powder puff ball of mycelia. In Sabouraud's glucose agar visible growth occurs on the

4th or 5th day but in Czapek's synthetic medium the growth is delayed till the 6th or 7th day. Subculture in Sabouraud's medium maintains the cultural character for a long time without pleomorphism. The growth is about 1 cm in 3 weeks. The character of the growth is cerebriform greyish brown colour covered with white powder. The growth increases to the size of 1.5 cm in 6 weeks. On glucose agar the growth is raised yellowish brown in colour. Under reduced oxygen tension in glucose agar the colour develops to a salmon pink when the tube is sealed with a rubber cap.

Microculture Terminal and intercalary chlamydospores are characteristic. Arthrospores in chain form when the growth occurs in glucose broth. Mycelial racquets are common and aleuriospores are absent or rudimentary (Fig 33).

Wood's light The culture shows a dull yellowish fluorescence.

Animal inoculation : Inoculations into human volunteers has been successful.

TRICHOPHYTON FERRUGINEUM (Ota) Langeron & Milocheritch 1930

Synonyms : *Microsporum ferrugineum* Ota 1921, *M. japonicum* Dohi and Kambayashi 1921, *M. aureum* Takeya 1925, *M. orientale* Carol 1928.

It causes ringworm of the hair in children in China. The infection was recorded in a European school boarding in India (Dey and Maplestone 1939).

Direct examination Infected hair shows spores having an arrangement like those of *microsporum*; they are about $3\ \mu$ in diameter.

Culture The growth is slow, convex, raised, glabrous and faviform with rusty colour and hence the name—*ferrugineum*. Irregular radial furrows

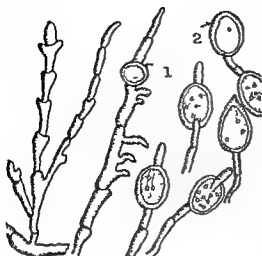


Fig 34—*T. mentagrophytes* (1) Intercalary and (2) Terminal chlamydospores

divide the growth into sectors. The growth may be covered with duvet in the margin (Pl XXI b)

Microculture The fungus is characterised by of terminal chlamydospores in long the are 20 to 30 μ which with their ends pointed. Atypical mycelial racquets rudimentary hyphae pectinate are often present. Atriospores are rare when present they are sessile and atypical.

Wood's light The infected hairs do not show fluorescence.

Animal inoculation Inoculations to laboratory animal are sometimes successful (Pl XXI d)

TRICHOPHYTON DISCOIDES

Synonym *Ectoichophyton verrucosum* Bodin 1902 *Ectoichophyton ochraceum* Sabouraud 1909 *Ectoichophyton album* Sabouraud 1909 etc

It primarily affects the cattle. It causes large spored ectothrix trichophytosis with formation of kemon in children and tinea barbae in adults. One case of kemon caused by this fungus has been recorded in India by Dey & Karoti (1955).

Direct examination Spores are seen in rows and are about 3 to 5 μ in diameter. The mycelia often show a beaded appearance. No mycelia are seen inside the hair (Pl XXII a)

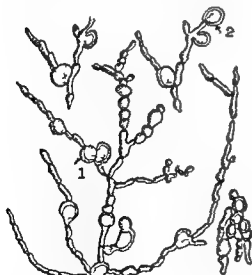
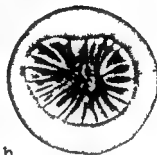
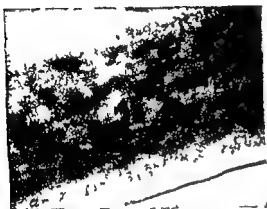


Fig 35—*Trichophyton discoides* with a) spores and (1) Intercalary and (2) Terminal chlamydospores

Cultural characters The growth is convex raised smooth and waxy and fissiform with irregular folds in the centre with radiating grooves at the periphery. It is characterised by the presence of a pigment when recently isolated (Pl XXII). But the pigment often disappears on subculture and may be completely lost in subsequent subcultures. Pleomorphism which is characterised by appearance of down on the surface of the glibrous growth is seen when subculture is delayed.

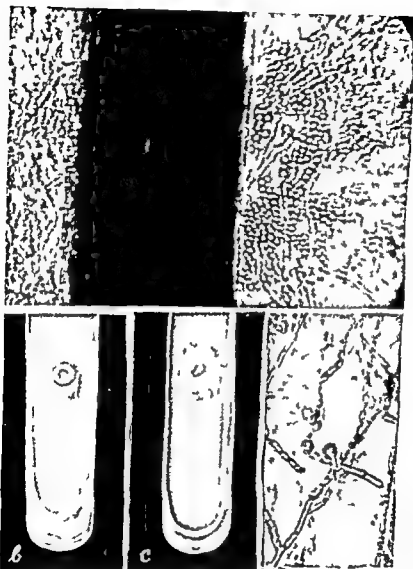
Microculture (Pl XXII) The mycelia show intercalary and terminal oval or round chlamydospores (Fig 35 Pl XXII d). Mycelial racquets are frequently present. Microconidia are absent.

TRICHOPHYTON FERRUGINEUM



(a) Hair showing small pores with mosaic appearance (b) Giant elongated faviform character (c) Macroconidia in microculture (d) Hair of the experimental animal showing temporary infection

TRICHOPHYTON DISCOIDES



(a) Petiole of the larva (1) in culture on Sabouraud's glucose agar (6 weeks old) (c) Subculture in 1% formalin (d) Terminal cilia in microculture

Wood's light : The infected hair gives a dull white fluorescence and is difficult to detect. In culture the growth appears to be dull white but clear.

Animal inoculation : Experimentally the fungus produces lesions in common laboratory animals.

GENUS EPIDERMOPHYTON

EPIDERMOPHYTON FLOCCOSUM (Harz) Langeron & Miloshevitch 1910

Synonyms : *Acrothecium floccosum* Harz 1871. *Trichothecium floccosum* Harz 1870. *Epidermophyton inguinale* Sabouraud 1910. *E. phlicarum* Nicolau 1913. *E. chaperformis* MacCarthy 1925. *Trichophyton inguinale* Sabouraud 1907. *T. intertriginis* Sabouraud 1905. *T. cruris* Castellani 1908. *E. cruris* Castellani and Chalmers 1910.

It invades the skin producing a condition of eczema marginatum of Herberich or ringworm of the groin commonly known as dhobie itch. But it may affect the glabrous skin of other parts of the body as well as the nail. It does not affect the hair.

Direct examination : Mycelia containing chains of spores are seen in a fairly large number. They are usually wavy in appearance (Pl XXIII a).

Cultural characters : It is a slow growing fungus. In Sabouraud's glucose medium the colour is lemon yellow or olive green with short white cottony duvet on the surface which are sterile. The centre of the giant colony is folded with radiating furrows (Pl XXIII b). Pleomorphism is characterised by formation of white duvet on the surface of the growth.

Microculture (Fig 36) Macroconidia are large clavate smooth thin walled and pluriseptate with 3 to 4 cells and 7 to 12 μ by 20 to 40 μ in size. They are often seen in clusters with rounded ends. Chlamydospores are seen in large numbers. Mycelial racquets are also

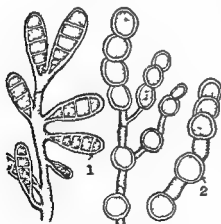


Fig 36—*Epidermophyton floccosum*
(1) Pluriseptate macroconidia with rounded ends (2) Chlamydospores

seen. Spirals if present are rudimentary in nature. Microconidia or pectinate hyphae are absent.

Wood's light : The colony appears dark olive in colour.

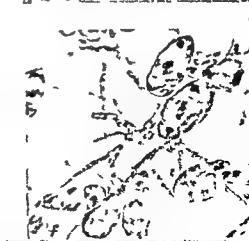
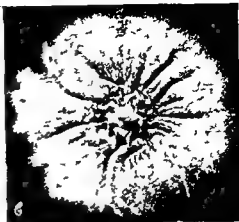
Animal inoculation : Laboratory animals are not susceptible.

TABLE VIII

DIAGNOSTIC FEATURES OF MICROSPORIUM TRICHOPHYTON AND EIDERMOLHYTON SPECIES

Description	Microsporium	Trichophyton	Epidermophyton
Colony morphology	Cottony or powdery aerial mycelia varying from white to buff colour	Cottony granular or glabrous (smooth and wavy) pigment may be present loss of pigment on subcultures	Powdery or velvety with radiating furrows on the surface and lemon yellow in colour
Microscopical Asexual forms	Racquet mycelia hyphae pectinate chlamydo spores rarely formed spores rare in spores like 1/2 con.	Racquet mycelia chlamydospores spiral hyphae nodular organs rarely hyphae pectinate In faviform types—favic channels and pectinate bodies in addition to racquets and chlamydospores	Racquets occasionally and chlamydospores frequently present
Microconidia	Small clavate 3 by 5 μ borne along the hyphae as also or fixed on facets	Numerous small clavate sub-spherical or spherical - by 4 μ borne along the hyphae (thyrsae) or in clusters (on granules) they are rudimentary or absent in faviform group	Absent
Macroconidia	Large spindle shaped thin walled when young but thick walled and tuberculate when old size 8 to 12 by 40 to 50 μ in length blunt plate but often with out set to in 1/2 and 1/3 They are pointed and contain fine hairs at the tip	The macroconidia shaped or cylindrical organs 4 to 6 μ by 10 to 50 μ smooth thin walled hyaline containing 3 or 4 setae In faviform groups macroconidia are absent	Large clavate septate smooth thin wall with rounded ends and often in clusters

PLATE XXIII
EPIDERMOPHYTON FLOCCOSUM



(a) Infected plant (b) Growth on Sabouraud and maltose agar (c) Macroconidia under low power (d) Chlamydospores (high power 43×8) (e) Macroconidia under high power (e) $43\times$ (f) 43×1

M. audouinii infection of the scalp and clinically it can be easily differentiated from black dot ringworm caused by trichophyton infection (Table IV). From the hairy scalp the infection may spread on the face, neck or trunk and called tinea circinata. These are small patches on the glabrous skin and produce small scaly irritable patches without the usual festooned margin (Pl XXIV).

Kerion formation is commonly seen in infections caused by *M. canis* and *M. gypseum* which are animal microspora.

Diagnosis *Clinical picture* Microsporum infection is essentially a disease of children especially during the school going age. The presence of one or more bald scaly areas exhibiting broken hair stumps sticking out in different directions is absolutely diagnostic.

Wood's light For further verification examination of the patient in a dark room under Wood's light shows fluorescence of the affected hair attacked by *M. audouinii*. In order to obtain satisfactory results the affected part should be cleansed with ether alcohol mixture. Wood's light is filtered ultraviolet rays through Wood's filter. The glass is made of sodium barium silicate containing about nine per cent of nickel oxide. The filter permits the passage of the spectrum of monochromatic ultraviolet rays in the region of 3650\AA° which produces the maximum fluorescence in case of *M. audouinii* infection.

Duckworth's chloroform test A few suspected hairs are placed on a glass slide and soaked with a few drops of chloroform. The test is positive when after the evaporation of chloroform the hair becomes chalky white in colour. A positive result is characteristic of microsporum infection in which there is a sheath of spores around the infected hair.

Microscopic examination The broken infected stumps of hair are mounted in a drop of sodium sulphide solution under a coverslip and the solution is heated without boiling. After a few minutes the hair is examined under the low power and then under the high power which shows a characteristic mosaic appearance due to arrangement of spores in groups. The spores are about 2 to 3 μ in diameter. Near the root of the hair Adamson's fringe may be seen (Pl XI b) indicating that infection is spreading in this part of the hair.

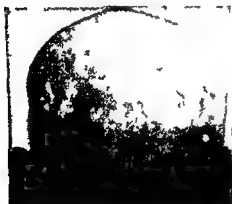
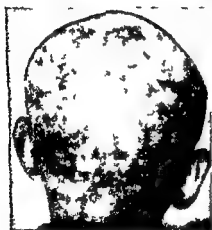
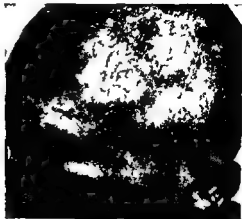
For the diagnosis of species study of cultural characters is necessary.

TRICHOPHYTOSIS OF THE SCALP

Trichophyton infection of the scalp is chiefly due to endothrix trichophyta. They cause black dot ringworm of the scalp in children.

Classification Trichophyta have been divided into three clinical groups according to whether the fungus infects the interior or the exterior part of the hair or both the interior and the exterior. When the infection is limited to the interior of the hair the fungus is called endothrix trichophyton. The spores of the endothrix trichophyta are larger than those of microspora and are about 3 to 5 μ in diameter e.g. *T. violaceum*, *T. tonsurans*, *T. accuminatum*. Those which infect the surface or the external part of the hair or the hair follicle are called ectothrix trichophyta e.g. *T. mentagrophytes*. The fungi that infect both the interior and the exterior of the hair are called ectoendothrix or neoendothrix.

PLATE XXV
BLACK DOT RINGWORM (*TRICHOPHYTON TONSURANS*)



Black dot ringworm of the scalp caused by *T. tonsurans*.
baldness caused by stumps of broken hairs

Note the small multiple areas of
To Face Page 92

PLATE XXVI
BLACK DOT RINGWORM (*TRICHOPHYTON VIOACEUM*)



Multiple patches of black dot ringworm of the scalp as used by T. and C. M.
To Face Page 93

But this is no longer considered as a separate clinical type because during the early part of infection an endothrix trichophyton may look like a neoendothrix

Ectothrix trichophyta have been further subdivided into those with small spores 3 to 5 μ in diameter i.e. *microides* e.g. *T mentagrophytes*. Those with large spores are called *megaspores*. The spores in this case are 5 to 7 μ in diameter e.g. *T rosaceum* *T discoides*. Ectothrix trichophyta are often of animal origin and show inflammatory reactions with kerion formation.

Etiology Three species of endothrix trichophyta may produce black dot ringworm of which one produced by *T violaceum* is the commonest in India (40.91 per cent of ringworm of the scalp) and is specially seen in Mohammedan children. *T crateriforme* common in France has been isolated from Anglo Indian and European children in India. *T sulphureum* is an allied organism common in England. This and *T accuminatum* have not yet been encountered in India.

Symptoms Black dot ringworm is characterised by small, multiple bald patches on the scalp which on close observation are seen to be studded with numerous black dots at the mouth of each hair follicle. This is due to the hair being broken off at the point of emergence from the hair follicle giving a characteristic appearance (Pl XXV XXVI). In a certain number of cases the skin may be affected specially on the face neck where multiple ringed patches of tinea circinata are seen.

Diagnosis Microscopic examination of the infected hair shows mycelial spores either oval or with square cut ends arranged in chains in the long axis of the hair. These spores are about 3 to 5 μ in diameter and are larger than those of microspora. It is very important to differentiate scaly and black dot ringworms which are tabulated.

TABLE IV
DIFFERENCE BETWEEN MICROSPORUM AND TRICHOPHYTON RINGWORM

	Microsporum (scaly) ringworm	Trichophyton (black dot) ringworm
Patches on the scalp	Only one or two big scaly patches	Multiple small black dot patches
Hair stump	Stumps are long and stick out in different directions. Grey sheath forms at the root of the hair.	Stumps are very small and flush with surface of the skin. No sheath is seen. They break easily at the time of pilation.
Microscopic	Spores are small (3 μ) and give a mosaic appearance.	Spores are large (5 μ) and give a beaded appearance along the long axis of the hair.
Culture	Shows characteristic growth of the species.	Shows characteristic growth of the species.

Kerion It is a suppurative condition of the scalp in cases of ringworm with inflammatory changes of the hair follicles. The primary infection is responsible

for the suppurative condition but secondary infection is often present. The inflammatory reaction is often beneficial to the host as the process is nature's attempt to get rid of the infection by suppuration and spontaneous epilation.

The infecting fungus may be *M. canis*, *M. gypseum* or *T. mentagrophytes* (Pl. XXVII). The condition of kerion is characterised by a boggy, inflammatory swelling of the ringworm patch of the scalp produced by a fungus of animal origin. Individual hair follicle shows folliculitis to begin with and this later progresses to an acutely inflamed patch with pus formation. Hairs in the patch become loose and can be easily pulled out with a pair of epilation forceps. As suppuration occurs there is a tendency of spontaneous cure by scarring and permanent baldness results due to obliteration of the hair follicles by the scar tissue.

Prophylaxis. Ringworm of the scalp is a highly contagious disease and is extremely common amongst school children. Epidemics may break out in boarding schools. All children should have careful examination of the scalp before admission into the school and also periodically when they join the school after vacations. Help of Wood's light is essential for this. As soon as any infection is detected, the child should be isolated and put under treatment. Hats, hair brushes, combs, etc. of patients and all contacts should be sterilised but when this is not possible these articles used by the patient should be burnt. The patient should wear a tight fitting linen cap through out the course of treatment. He or she should not attend the school till completely cured. In a boarding school the child should be segregated.

Treatment. The main principle of treatment is to get rid of the infected hairs by a process of epilation. Epilation is followed by application of mild antiseptic ointment which contains non irritant fungicidal agents. The layer of ointment acts on the spores of the fungus and also prevents the spread of infection by hair. The ointment should not be strong enough to cause too much irritation or inflammation of the affected part. Ointments that are usually used are 5 per cent ammoniated mercury or 1 per cent thymol or 0.1 per cent phenyl mercuric nitrate or an ointment containing 0.5 per cent each of iodine crystal and cinnamon oil. Irritant applications like croton oil, turpentine or chrysarobin are unnecessarily hazardous. Loose hairs may be best removed by shampoos or a liquid soap. Rubbing too vigorously or too frequently may break the hair instead of making them loose. In infections with fungi of animal origin like *T. mentagrophytes*, *M. lanosum* inflammatory reaction is severe and as a result there is a tendency of suppuration and spontaneous cure by scarring of the hair follicle. In these cases prognosis is good and all that is required is the application of a mildly antiseptic ointment.

ANTISEPTIC COMBINED WITH HORMONE THERAPY. In a study by Lewis Hopper and Reiss the authors found oestrogen and androgen to be quite effective *in vitro* against ringworm fungi but clinical results were poor when such agents were applied locally to areas of infection. Gonadotropic hormones were tried by Ghosh and Dey (1945) in 23 cases of ringworm of the scalp combined

with local application of fungicidal paints containing thymol and cinnamon oil in tincture iodii mitis or 0.1 per cent ointment of Phenyl mercuric nitrate. The use of the gonadotropic hormone has been limited apprehending that precocious puberty might be initiated specially in girls. Oestrogenic hormone like stilboestrol is given in doses of 1 mg three times daily for 20 days or until the child exhibits symptoms of toxicity such as inflammation of the breast vomiting etc. It has been found that combined treatment is better than either hormone or antiseptic ointment alone. Those who are interested are referred to the original paper.

EPILATION This may be rendered in one of the following ways. The hair should be cut short and the scalp should be examined with Wood's filter from time to time for the progress of the case.

MANUAL EPILATION This is feasible when the patch is small and single. The infected hairs as revealed under Wood's light in a dark room are pulled out with a pair of epilation forceps. The process is repeated twice weekly and the patches are covered with the antiseptic ointment after epilation. On other days the patient can apply soap or shampoo after which the ointment is applied. The patient should wear a tight linen cap which should be boiled every day.

EPILATION BY IRRITANT DRUGS Local application of irritant drugs produces acute inflammation and loosening of the hairs which are then easily pulled out e.g. croton oil 2 per cent chryserobin in acetone. But in such applications there is danger of scarring and permanent baldness. Suppuration must however be stopped before the application of X ray treatment.

EPILATION BY THALLIUM ACETATE Thallium acetate administered orally in therapeutic doses loosens hair roots so that all the hairs can be easily pulled out after 18 to 21 days. The drug has the disadvantage that the therapeutic and toxic doses are close to each other so that there is always a risk of using it. The therapeutic dose is 8 mg per kilo body weight given as a single dose in children up to 10 years. The toxic dose is 8.5 mg per kilo body weight.

Mode of administration The child must be accurately weighed in a nude state and the weight is recorded. The dose is calculated according to the body weight and thallium acetate is administered as a single dose in a cupful of sweetened water. Hair begins to fall after 3 weeks. The therapeutic action of the drug is due to the selective action through the sympathetic trophic nerves. Common toxic symptoms are pain in the muscles and joints, convulsions of choreic nature, albuminuria, gastrointestinal disturbances etc. Adhesive plaster is applied all over the scalp on the 18th day after the draught and complete epilation is produced by taking off the plaster cap on a subsequent date. Fungicidal remedies should then be applied to eradicate the infection thoroughly and completely before regrowth of the hair which commences after four weeks.

EPILATION BY X RAYS Total epilation with X rays followed by the application of antiseptics is the standard method of treatment. For X ray epilation the method of Adamson-Kienbock is followed. In this the scalp is divided into five areas namely frontal, vertical, occipital and one over each ear and

approximately a dose of 370 units of X rays are administered to each area. This treatment does not kill the fungus but the hair papillae are devitalised temporarily and within 21 days hairs fall out including the infected ones containing spores and the fungus. The scalp still remains infected and antiseptic ointment as mentioned before should be applied daily for this purpose along with the application of soap and wash.

The cure in such cases should be carefully observed and examined under Wood's light so that no area remains untreated or becomes newly infected.

Contraindications of X rays Impetiginous pustular lesions should be treated and cured before the X ray exposure otherwise the lesion may spread on the whole scalp. Kerion cases should not be treated with X ray.

Danger of X rays A permanent baldness may occur after an over exposure. This should always be done by experts with an indemnity bond from the guardian of the patient. Ulcers may be aggravated and may cause deep scars with permanent baldness.

Treatment of Kerion Thick crusts are removed with starch and boric poultice daily for several days till the scalp is clean. Loose hairs are removed with a pair of epilation forceps. When the part is fairly clean lotio triple dye is painted twice a day on the patch.

TINEA BARBAE

Synonyms —Tinea sycosis hyphogenic sycosis etc.

Definition This is an infection of the beard areas of the face and neck caused by ectothrix trichophyta or microspora of animal origin producing inflammatory lesions and pustule formation. Cases have been reported from Europe and America; two cases have been recorded in India by Dey et al (1946) caused by *T. gypsum* (Pl XXVII d).

Pathogenesis The disease may be transmitted from man to man through direct contact or shaving in barber's shop. The infection may be contracted also from infected animals like horses.

Signs and symptoms The disease starts on a localised area of the beard region as red itchy acutely inflamed scaly patch with formation of pustules at the root of the hair giving a boggy appearance. Suppuration takes place in the hair follicles which remain widely gaping. The hair remains loose in the follicle and the infected hair can be easily pulled out with a pair of epilation forceps. The affected patch becomes raised inflamed and painful. In milder cases the inflammatory reaction may be less severe or only small areas of scaly patches are seen. In an advanced case almost the whole of the beard area may be involved in patches with groups of suppurating hair follicles. The upper lip is not usually involved but the infection may spread here also involving the moustache area.

Differential diagnosis Tinea barbae should be differentiated from sycosis barbae the latter being staphylococcal in origin. Sycosis barbae produces superficial pustular folliculitis. There is pus in each follicle with erythema.

PLATE XXVII

LESIONS CAUSED BY

TRICHOPHYTON MENTAGROPHYTES



(a) (b) (c) (d) (e) (f) (g) (h) (i) (j) (k) (l) (m) (n) (o) (p) (q) (r) (s) (t) (u) (v) (w) (x) (y) (z)

PLATE XXVIII RINGWORM OF THE GLABROUS SKIN



(a) (b) (c) and (f) caused by *T. purpurum* (c) Extension from ringworm of the back caused by *T. violaceum* (d) Eczema marginatum of Hebra caused by *Epidermophyton floccosum*

and induration around the hair follicle. The whole of the beard area is affected instead of patches as in tinea barbae. Hairs are not loose and cannot be easily pulled out.

TABLE V

DIFFERENCE BETWEEN TINEA BARBAE AND SYCOSIS BARBAE

Tinea barbae	Sycosis barbae
DISTRIBUTION The infection is localised in patches single or multiple	The infection is generalised infecting all the hair follicles
ORGANISMS Trichophyta or microspora of animal origin	Staphylococci
LESION Kerion like boggy patches of inflamed areas. Hair follicles are gaping and hairs are loose and pulled out easily	Hair follicles are affected with superficial pus formation and erythema and induration. Hairs can not be pulled out easily
MICROSCOPIC EXAMINATION The fungus can be demonstrated in the hair	Hairs do not show any fungus

Treatment In a very early case when the infection is limited to the skin and has not affected the hair follicles local application of Whitfield's ointment or application of tincture iodine is quite effective to remove the infection. When the hair follicles are affected a preliminary application of hot boric compress or starch boric poultice will help removal of the crust. Manual epilation is easy as the hairs are loose in the widely gaping hair follicles. When the part is clean triple dye or tincture iodine may be applied twice daily. Phenyl mercuric nitrate ointment (0.1 per cent) or 5 per cent ammoniated mercury may be applied at night. Sodium propionate (10 per cent solution in alcohol) may be substituted for ammoniated mercury ointment. In persistent cases X-ray epilation of the affected parts is advised. At least two microscopic examinations should be negative before the patient is declared to be cured.

RINGWORM OF THE GLABROUS SKIN

This is an infection caused by a group of fungi and characterised by ringed vesicular lesions of the skin specially on the inguinal region, loin, back, hands and feet or soddening of webs of toes, exfoliative and hypertrophic lesions of the palms of the hands or soles of the feet. They produce severe itching followed by weeping, crust formation and exfoliation. The lesions spread by peripheral extension to a large extent.

Etiology The causative organisms are *Epidermophyton floccosum*, *Trichophyton rubrum*, *T. interdigitale* and other trichophyta and rarely microspora.

AGE The disease is commonly seen in adolescent and young adults and may persist throughout the whole life. It is very rare in children below 12 years and this is accounted for by some due to the presence of thymus secretion which inhibits the growth of the fungus in the glabrous skin.

SEX The infection is commoner in males than in females.

FRICTION Friction of dhoti in the loin area chafing in the intergluteal fold and inguinal region remove the protective horny layer of the skin and predispose to infection. Constant washing with alkali soap has the same effect by removing the superficial horny layer.

TEMPERATURE The fungus grows well in hot and humid weather and the disease remains quiescent during the winter months.

MOISTURE Moisture is necessary for the growth of the fungus and perspiration in the hot weather keeps up the moisture. In the inguinal axillary and interdigital areas the skin becomes sodden with perspiration as there is lack of proper evaporation in these areas. Sweat washes the acid mantle of the skin which contains propionic and other aromatic acids and protect the skin from infection. Thus an ideal substrate is provided for the germination of spores of the fungus and high atmospheric humidity augments the condition.

SAPROPHYTIC LIFE OF THE FUNGUS The fungus passes a saprophytic life outside the human body in moist earth, wood, damp leather and other organic substrates like decaying organic and vegetable matters. Hence people walking bare footed or athletes are prone to this infection and in them growth of the fungus is favoured by moisture in the interdigital regions. The fungus may thrive on seats of comode, bath mats, coir mats etc.

Pathology In culture it can be demonstrated that the fungus has roots, surface runners and aerial hyphae bearing spores. The roots of the fungus grow downwards passing in between the prickly cells as far down as the papillae to open up the lymph spaces and derive their nutrition. This results in formation of vesicles at the spreading margin of the lesion. This is associated with intense itching due to irritation of the nerve endings until the tension is relieved by the rupture of the vesicle accompanied by serous exudation. The serous exudate dries to form a thick scab containing the vegetative fungi and arthrospores at the spreading margin of the lesion. Serum usually inhibits the growth of ordinary bacteria but streptococci may grow in the clear secretion causing secondary streptococcal dermatitis. (For details see Acton and McGuire 1927.)

Ringworm of the glabrous skin may be described under (1) *Tinea circinata* (2) *Tinea corporis* (3) *Tinea cruris* (4) *Tinea axillaris* (5) *Tinea pedis* etc.

Tinea Circinata These are characterised by round or oval patches usually secondary to lesions on other parts of the body. The lesion may spread from the scalp and is caused by animal *Microspora* or *Trichophyta*. *Microsporum audouinii* infection of the glabrous skin is usually seen in children characterised by round or oval scaly patches without any typical ring formation. The lesions are often seen on the neck, forehead, face or shoulder (Pl. XXVIII).

Trichophyton infection of the glabrous skin may be caused by an endothrix or ectothrix trichophyton. The lesion produced by *T. violaceum* in children are often generalised multiple and scaly in character (Pl XXVIII). They may occur on the glabrous skin of the adult without any scalp lesion. The lesions are scaly in character. Lesions produced by ectothrix trichophyta show definite circinate patches the margin of which is raised and well circumscribed. The spreading margin is often associated with vesicle and pustule formation.

Tinea corporis Ringworm of the glabrous skin often occurs on the trunk and extremities and spread from the primary affection of the crural region (See tinea cruris). In infections caused by *T. rubrum* in India lesions spread extensively on the body with a typical purplish colour of the lesion.

Tinea cruris The classical form of the disease is seen in infection caused by *E. floccosum*. The infection was originally described by Hebra in 1860 as eczema marginatum. It is usually initiated by friction caused by underwears and this is why it is often called Dhobi itch. The crural region may show ringworm caused by *T. gypsum* var *interdigitale* and *T. rubrum*. The latter infection is common in India and very persistent. *T. gypsum* var *interdigitale* usually spreads from a nidus present in the web between the toes where it produces athlete's foot but in case of *T. rubrum* infection of the foot nail or the inguinal region forms the nidus from where infection spreads on to other parts of the body.

In the genitocrural region the lesion starts as a well circumscribed ringed lesion with a raised margin studded with small vesicles at the periphery. The patch is severely itchy and the erythematous vesicles are soon broken and dry scabs form at the margin. Streptococcal infection is apt to occur at this stage giving an acute eczematous condition of the lesion. In an established case the lesions are symmetrically distributed showing a festooned raised grate margin. It spreads peripherally on the inner margin of the thigh, scrotum, perineal and intergluteal regions and rarely to the skin over the penis. Secondary infection by streptococci and staphylococci produce impetiginous scabs. Hair involvement is unknown.

Tinea axillaris These lesions which are clinically similar to tinea cruris are seen in the armpit and both the conditions namely tinea cruris and tinea axillaris are often seen in the same patient at a time.

Tinea pedis The common causative organisms are *T. rubrum*, *T. gypsum* var *interdigitale* and *E. floccosum*. Three clinical types are generally seen namely (1) Interdigital type (2) Vesicular type (3) Hypertrophic type in the sole of the foot.

Interdigital type This is the commonest and the most important type and commonly known as athlete's foot, mangoe toe or Hongkong foot etc. The lesion is characterised by sodden whitish appearance of the skin between the fourth and fifth toes and forms the nidus of infection. The infection is persistent and is maintained at this site for a long time with acute exacerbation in the summer and rainy seasons (Pl XXIX b).

Vesicular type This often originates from the margin of the foot and appears on the instep or back of the foot where the skin is comparatively thin. At these sites they form vesicles in groups and a definite margin may be present in some cases. The lesion shows an acute inflammatory reaction which is painful and it is often followed by pus formation. The fungus that is isolated from this type of lesion is *T. gypseum* or *T. interdigitali* (Pl XXIX c).

Hypertrophic type This is seen in chronic cases of ringworm caused by *T. rubrum*. In this type the horny layer of the sole including the heel and side of the foot are thickened diffusely or in hypertrophic patches forming plaques. The surface of the hypertrophic area shows exfoliation cracks and fissures (Pl XXIX a and e). They should be differentiated from similar hypertrophic lesion produced by *Nocardia keratolytica* (Pl IV Fig 2) yaws and syphilis. Such a lesion is apt to occur sometimes on the dorsum of the foot also (Pl XXIX d).

Complications Secondary infection often takes place producing weeping eczema caused by streptococcal infection or a condition of cellulitis. Phytides are occasionally seen on the palms and soles as a result of sensitisation caused by spores escaping into the blood stream from the site of primary lesion but fungi are absent in the id lesions.

Melanoleucoderma and ringworm fungi There is a condition of melanoleucoderma as a late syphilitic condition characterised by hyperpigmentation of palms and soles giving an appearance like that of Pinta. Infection with *T. rubrum* is often associated with it and the fungus can be isolated in those cases. It is not only present in the tropics but one such case was seen by the author in St John's Skin Hospital London (1950). The fungus in that case was found to be *T. sulphurum* instead of *T. rubrum* (Pl XXV).

Diagnosis Ringed lesions in the groin, axilla or other parts of the body with scaly appearance and associated with intense itching are very much suggestive of ringworm but ringworm like lesions in the neck of adult persons is almost always due to seborrhoeic condition rather than ringworm.

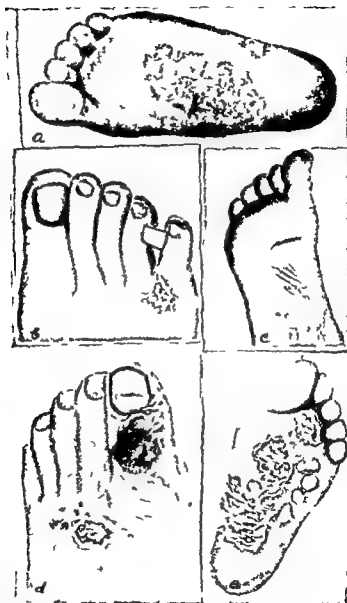
In interdigital affections the skin is sodden but on the instep vesicular lesions are characteristic. This condition must be differentiated from pompholyx of the hands and feet caused by sensitisation. Diagnosis should always be confirmed by the presence of fungi by microscopic and cultural examination of the material. In pompholyx and other id reactions the fungus is absent and fungus infection is ruled out if the trichophytin test is negative.

MICROSCOPIC EXAMINATION The part for scraping is first cleaned with a mixture of equal parts of ether and alcohol and then scraped with a sterile (flamed) Paget's knife. The scraping material is collected between a pair of sterile slides. A part of the scraping material is transferred on to another slide and a coverslip preparation is made with sodium sulphide solution. The slide is gently heated without boiling and the preparation is ready for examination when there is complete keratolysis.

While examining the scales care must be taken not to be confused with fungus like structures the so called mosaic fungus. This is an artifact due

PLATE XXIX

TINEA PEDIS



(a and e)—Hypertrophic patches (b)—Interdigital lesion (c)—Volar lesion (d) Hypertrophic lesion with ulceration

PLATE XXX

MELANOLEUCODERMA



Melanoleucoderma of the hand. Note hyperkeratosis of the skin & mottled from all of them.

To Face Page 101

to deposit of cholesterol between the cells of the epidermis. These deposits form structures like true mycelia but unlike mycelia they are irregular in their size and contour. They are always found in the intercellular space and do not traverse across the cells whereas true mycelia always lie across the epithelial cells (Fig. 29 c p. 83).

CULTURE: Infected materials can be cultured on Sabouraud's medium in test tubes but for the study of colony characters giant colonies are obtained in flasks containing Sabouraud's proof medium with maltose agar.

Prophylaxis: The disease is apt to recur and for this reason prophylaxis should be strictly observed. The nidus in the interdigital or crural regions should be treated properly and radically cured. Walking bare footed is strictly avoided. As there is chance of contamination underwears should be boiled every day or as frequently as possible. Public baths and commodes are sources of contaminations and these should be avoided as far as possible. In the rainy season shoes may become mouldy. The shoes should be disinfected with formalin spray at regular intervals and put in the sun. Socks should be changed and boiled or disinfected every day. Feet should be kept dry using sulphur and camphor powder. Other flexural and intertriginous regions of the body should be similarly treated. Vinegar or 3 per cent acetic acid or powder containing propionic acid or undecylenic acid may be applied in the foot and intertriginous regions.

Treatment: Ringworm is a chronic disease but in the genitocrural and other regions where the skin is thin acute exacerbation due to streptococcal infection is often seen. In the acute condition evaporating lotions Goulard's lotion, lotio calamine or 1/4000 solution of potassium permanganate may be applied locally. But when the infection has further advanced lotio acriflavine (1/5000 aqueous) may be applied on a gauze soaked with the lotion intermittently for two to three hours three or four times a day. This is applied in such a way that the part is not sodden. When the oozing has stopped unguentum zinc or borovaseline should be applied. Secondary infections in the interdigital part of the toes with cellulitis is best treated with application of Pot. permanganate lotion followed by application of lotio triple dye. When dry this may be followed by application of Castellani's paint.

In the chronic condition unguentum Whitfield is the best application particularly on the thin sensitive skin like those of scrotum, groin or face. In the groin as a routine measure undecylenate powder (10 per cent zinc undecylenate) or sulphur and camphor powder should be applied in the day time twice or thrice daily and at night application of Whitfield's ointment is advocated as a routine. Dithranol ointment cures tinea more quickly than other remedies but there is a great danger of irritation from the drug. For sensitive areas it should be used in a concentration of 0.5 per cent but in other chronic or resistant cases it may be used in a concentration of 1 to 2 per cent. On the body, hands, feet and hypertrophic patches on the sole of the foot ringworm paint is an excellent remedy. One application a day is quite effective.

unguentum Whitfield may be applied in those areas at night. In areas of secondary infection causing pyoderma unguentum hydrarg ammon dil may be applied to get rid of the secondary infection before Whitfield's ointment is applied on the part.

TINEA UNGUINUM (Ringworm of the nail)

Ringworm of the nail is caused by a group of fungi and the infection is often secondary to ringworm of other parts of the body. The infection is seen to involve nails of both fingers and toes as a result of extension of lesion from the neighbouring areas but the finger nails may be contaminated by scratching the body.

Pathogenesis: The disease is very common in India. It is caused by *Trichophyton* and *E. floccosum*. The commonest trichophytes in India are *Trichophyton rubrum* but *T. interdigitale* or *T. violaceum* are rarely seen.

Signs and symptoms: The disease commonly starts on the free border or the lateral margin of the nail plate. The infection may be due to direct extension of the lesion from the surrounding parts or as a result of scratching a ringworm patch on the body. The spores are usually lodged under the nail plate and mycelia proliferate from these spores causing a greyish white mass consisting of keratin and mycelial filaments which raise the nail from its bed. The infected nail becomes hypertrophic, opaque, lustreless and brittle, yellowish white in colour. The nail bed is gradually involved and the nail shows a typical moth eaten appearance (Pl. XXXI).

Diagnosis: Psoriasis may involve the nail plate very often resembling ringworm of the nail but signs of psoriasis on other parts of the body would at least indicate the possibility of the affection caused by psoriasis. Microscopic examination of the nail under a sodium sulphide preparation will clinch the diagnosis in a case of ringworm. For this the coverslip preparation of the material with sodium sulphide solution is kept for about three hours with heating of the solution at intervals. After that period the material becomes soft and is pressed gently under the coverslip to spread it. It is then examined under the low power and confirmed under the high power. Mycelia often show a beaded appearance due to formation of arthrospores.

Culture of the material is best done in Sabouraud's medium with gentian violet. This prevents the gross contamination and creates a favourable condition for the growth of the fungus.

Treatment: No local treatment is satisfactory except avulsion of the nail under nitrous oxide anaesthesia. Thorium varnish has been claimed to have given very good results. Application of Castellani's paint twice daily when continued for about six months may cure the condition in some cases. For this the nail is scraped to such an extent that the affected tissue is almost completely removed before the paint is applied.

PLATE XXXI

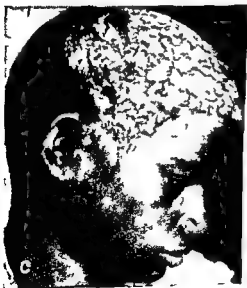
RINGWORM OF THE NAIL AND HANDS



The figure shows typical moth-eaten appearance. The skin on the dorsal aspect of the hand shows a condition of hyperkeratosis (lower left). The commonest organism in India is *Trichophyton* (T. b. m.).

PLATE XXXII

FAVUS (Scalp)



Favus caused by—(a and b) *Achrocybe* (c and d) *Aspergillus*

FAVUS (Tinea favosa)

Favus is an infection of the hair and skin caused by *Achorion schoenleini*. In Japan it is caused by *A. schoenleini* and *A. schoenleini* var *mongolica* but in India favus is caused by *A. actioni* and an organism allied to *A. schoenleini* var *mongolica* (unpublished). It may affect a person of any age but children are usually victims of this disease. It usually invades the scalp but glabrous skin, nails or mucous membrane may be affected. The fungus gives a typical mouse like odour. In India the disease is commonly seen in Kashmir, Punjab and Rajputna but does not occur in the eastern and southern parts.

Symptoms. On the scalp the lesion begins as small yellow sulphur coloured cup shaped discs around the hair follicle called scutula. The concavity of the cup is upwards. When a disc is removed it leaves an oozing ulcerated area which is red with seropurulent discharge. The scutulum increases in size and coalesce with the adjacent ones. Thus in an advanced case the whole scalp appears to be covered with a thick yellow coloured mud plaster (Pl XXXII). The lesion spreads from the scalp to the body and forms scutula along the lanugo hairs. Nails are similarly involved showing the same type of thick deposit of the fungus mixed with dry secretion, debris, epithelial cells and leucocytes.

Diagnosis. Kerion may be mistaken for favus. When the scutulum is dissolved in sod sulphide solution and examined under the microscope it is found to contain a network of mycelial threads in which there are epithelial debris and pus cells. In the hair which pierces the scutulum mycelia are arranged along the long axis and hence the hair splits longitudinally and does not break.

Prognosis. In the scalp it is very difficult to eradicate the disease but favus of the glabrous skin is more amenable to treatment.

Prophylaxis. Favus is highly contagious and complete segregation of the patient is imperative. Hats, turbans or other head dresses should be burnt and tight fitting washable cotton caps must be worn and boiled daily for disinfection till the patient is completely cured.

Treatment. Scutula are removed with 10 per cent salicylic acid or 2 per cent mercuric oleate ointment. Epilation of the hair with X rays is advisable and the scalp is then treated with thymol iodine paint. Chances of baldness resulting from the disease are much greater than from X ray treatment. If the nail is affected it should be removed and the nail bed is treated as for ringworm of the nail.

TINEA IMBRICATA

Synonyms. Tropical ringworm. Tokelau and other local names.

Definition. Tinea imbricata or tropical ringworm is caused by *Trichophyton concentricum* and characterised clinically by the presence of extensive, flaky tissue paper like concentrically arranged scales.

History The disease was first described by Dampier in 'Voyage round the world' published in 1789. Tilbury Fox described the disease in 1874 as Tokelau ringworm but he considered the fungus to be identical with that of European ringworm. Manson (1879-82) was the first to describe the disease in China in any detail and he gave the name *tinea imbricata*. He considered the fungus to be a non cultivable trichophyton to which Blanchard gave the name *Trichophyton concentricum*. Castellani in Ceylon (1910-11) was the first to cultivate the fungus and proved it to be the causative organism by successful inoculation in human volunteers.

Etiology and pathogenesis The infection is found in all age groups including children. Both the sexes are equally affected. The fungus infects the glabrous skin but hair or the hairy scalp is not affected. The fungus grows between the superficial layers of the epidermis forming a felt of interlacing mycelia which detach the horny and granular layers from the rete.

Geographical distribution The disease occurs commonly in the Malay Peninsula, South Pacific Islands, Southern China, India, Ceylon, South Africa and Central America. In India it is very common in the aboriginals and tribal population of Assam residing in the submountainous regions, namely the Mikirs, Garos, Khasis, Jaintias and Aborhs (Dey and Maplestone 1941) and rarely in the plains. The disease was described by Acton and Ghosh (1934) in a Bengali inhabitant residing in the plains at the foot of Garo hills.

Signs and symptoms The disease begins either in the trunk or in any of the limbs as a round or oval pigmented macule which is very irritable. The macule soon breaks in the centre forming a ring with the scale attached to their outer margin. The ring extends at the periphery and increases in size and another similar macule starts and a ring forms inside the first one. In this way a large patch is formed containing several concentric rings of scales. The arrangement of the scales has been very aptly compared by Manson with ripples produced by a stone thrown into a pool of water. Similar lesions follow on other areas and several systems or concentric rings appear which overlap one another. A fully developed lesion presents a system of parallel wavy lines which at the first glance creates an impression that a complicated series of figures has been tattooed on the skin. The undulating parallel lines appear like the imbrication of tiles on a roof and hence the term *tinea imbricata*. The flaky scales often stick out like tissue paper in such cases. The entire skin surface of the body may be affected except the axilla, groin and scalp, palms of the hands and soles of the feet. The nails when affected become brittle and friable. Irritation is usually severe but there is seldom any secondary infection or inflammatory changes (Pl. XXXIII).

Diagnosis The disease may be diagnosed clinically by the concentric or parallel rings of scales which often resemble ripples when a stone is thrown in a pool of water. The body is extensively involved in this condition. The finding of mycelial elements on the scales clinches the diagnosis. The

PLATE XXXIII

TINEA IMBRICATA



Concentric rings are seen in the upper left and parallel wavy lines in the lower left figure

PLATE XXXIV

MYCETOMA CAUSED BY *GLENOSPORA SEMONI*



- (a) Mycetoma of the foot (b) Culture on Sabouraud's agar (3 weeks old)
 (c) Micro culture showing elongated spores

fungus can be demonstrated in scales in a coverslip preparation with sodium sulphide solution. Culture is necessary for identification of the species but primary culture does not give uniformly positive result unless the material is properly selected.

Treatment No satisfactory treatment of *tinea imbricata* is known as yet. Strong keratolytics like 10 per cent chrysarobin or 5 per cent each of sulphur precipitate and salicylic acid in vaseline or 2 per cent derobin in an ointment base show a very good effect but results are temporary and lesions reappear within a short time. Castellani's paint has been recommended and this may be supplemented with Whitfield's ointment. A pigment containing resorcinol—dr 1 acid acetic—dr 1 and tincture benzoin co. oz 1 may be applied with some benefit. Ringworm paint is more effective but may irritate the tender skin.

Dermatophytides (id or ide lesions)

Dermatophytides are eruptions due to dissemination of mycelia spores or any allergen produced by the fungus from a primary focus of infection in the body followed by sensitisation of the skin.

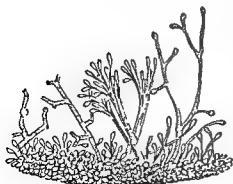
Various clinical types have been described, namely: papular, vesicular, pustular, eczematoid or rarely of the type of frank erythema multiforme. The commoner is the lichenoid type in which groups of conical follicular papules appear symmetrically on the trunk and extremities. Fungi are present in the primary focus but can not be demonstrated in the ide lesions.

Dermatophytides usually occur in acute lesions caused by ringworm fungi of animal origin, e.g. *M. gypsum*, *M. canis* and *T. mentagrophytes* and sensitisation can be demonstrated by a positive reaction to trichophytin. Epidermophytides may be produced by *E. floccosum* in the form of pompholyx of the hand originating from a primary focus in the foot.

Treatment The treatment of dermatophytides is symptomatic. They disappear spontaneously when the primary focus is treated.

GLENOSPORA

GLENOSPORA GRAPHII Siebermann 1889



Glenospora are characterised by large aleuriospores $10\ \mu$ or more in diameter. Mycelia are white at first and later dark and brownish in colour. The filaments are septate 2 to $3\ \mu$ in diameter, ramified. Fertile hyphae are erect with terminal conidia which are small ($5\ \mu$ or less) ovoid with smooth surface, greyish brown in colour (Fig. 37). They grow in ordinary media, gelatin is not liquefied.

G. graphii has been found in cases of otomycosis and keratomycosis.

Fig. 37—*G. graphii* showing terminal conidia

GLENOSPORA KHARTOUMENSIS Chalmers and Archibald, 1916

G. khartoumensis was found in a case of black mycetoma at Khartum in Anglo Egyptian Sudan

Morphology They show branched septate hyphae 2.8 to 14 μ in diameter. The hyphae are darker in old cultures. They form thick walled dark coloured chlamydospores (14 by 11 μ) forming black masses on the surface of potato infusions and nutrient gelatin. Aleuriospores are seen acropleurogenously on the surface growth of old potato infusions. They are 5 μ or less in diameter.

Culture The optimum temperature for growth is 30°C. It grows well in carrot as well as in glucose, maltose or nutrient agar. It does not liquefy coagulated serum or gelatin. In litmus milk it grows well and forms a silky precipitate. On maltose agar it gives a typical dusky drab growth and with a central elevation with radiating furrows and fringes at the margin.

Pathogenicity It causes African black mycetoma in man but inoculations have failed in laboratory animals under different experimental conditions by various routes.

GLENOSPORA SEMONI Chalmers and Archibald 1917

This organism was isolated by Semon from a case of black maduromycosis occurring in an Indian soldier serving in France. It has been recently isolated in India by Kakoti and Dey 1956 from a case of black mycetoma.

G. semoni closely resembles *G. khartoumensis* and may be considered synonymous with the latter. It slightly differs from *G. khartoumensis*. On maltose agar the culture is characterised by a smooth surface instead of a central depression or knob with radial furrows and fringes at the margin (Pl. XXXIV Fig. C).

Pathogenicity It causes Asian black mycetoma of the foot in man.

GENUS ACLADIUM Link 1803

Suborder Aleuriosporineae, Family Aleurismaceae, tribe Aleurismate

In culture the mycelia are thin and pile with accladium type of aleuriospores which are appendiculate and borne pleurogenously.

ACLADIUM CASTELLANI Pinoy 1916

The organism was found by Castellani in Ceylon and Greece. It has also been reported from China and Malay States. It causes ulcers anywhere in the skin with furuncles or nodules or bronchoaccladiosis (Castellani).

Culture Colonies are round. Mycelial filaments are about 2 μ in diameter. The chlamydospores are terminal (acrogenous). Aleuriospores are 4 by 3 μ , often lateral (pleurogenous) and rarely terminal (acrogenous) (Fig. 38).

Treatment The lesions are not spontaneously cured but improvement occurs when ulcers are treated with potassium iodide per mouth.



Fig. 34.—1. Castellani (a) accladium (b) development of mycelia (c) chlamydospores

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CHAPTER X

SUBORDER SPOROTRICHINEAE

SPOROTRICHOSIS

Definition Sporotrichosis is a subacute or chronic granulomatous lesion of the skin caused by *Sporotrichum schenckii* characterised by gummatous ulcers of the skin and subcutaneous tissues bones and viscera including the central nervous system. Spread occurs by extension along the lymphatics and rarely by the haematogenous route.

History Schenck (1898) first isolated and described a fungus in Baltimore from a patient with a subcutaneous abscess on the arm and placed it in the genus *Sporotrichum*. Hekton and Perkins (1900) reported a second case in USA and called the organism *Sporothrix schenckii*. Beurmann and Ramond (1903) found a similar infection in France and Matruchot and Ramond (1905) called the causative fungus *Sporotrichum beurmanni*. Several other species have been described from many parts of the world but they are now considered to be variants of *S. schenckii*.

Geographical distribution The disease is common in USA and France. du Toit (1942) reported 650 cases of sporotrichosis occurring in the gold mines of South Africa. Twelve cases were recorded in the skin out patient department of the School of Tropical Medicine Calcutta during a period of 15 years and of these detailed studies of two cases were reported by Ghosh (1932) and Panya et al (1947).

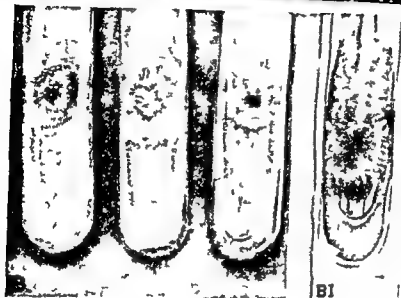
The fungus is widely distributed in nature and it has been recovered from plants and timbers in mines by du Toit. Spontaneous infection occurs in horses rats cats dogs and rabbits. Meyer (1915) found that the organism was present in the hair and skin of healthy horses coming in contact with the diseased ones.

Etiology The disease may occur at any age. Males are more frequently affected than females. The disease is specially seen amongst farmers gardeners mine workers and horticulturists. Persons coming in contact with horses and cattle are liable to contract the disease. Man may be infected through wounds coming in contact with plants and du Toit observed that mine workers might contract the disease from infected timber.

Organism The causative organism of the disease is *S. schenckii*. In France *S. beurmanni* was found to be responsible to cause sporotrichosis. Panya Dey and Ghosh (1949) described a new species and called it *S. tropicale*. There has been a considerable discussion about the validity of the plurality of species on the basis of growth characters in different media sugar reactions disposition of conidia etc. Davis has however shown that both the pigment and spore formation are too variable to be used as points for differentiation because both these depend on nature of the medium and frequency of subculture. Meyer and Aird (1915) again showed that different isolates vary in their biochemical reactions and most strains liquefied gelatin slowly. Therefore workers are in favour of recognising a single pathogenic species namely *S. schenckii*.

PLATE XXXV

SPOROTRICHOSIS



(A) Gummatous ulcer of the right arm and for arm (B) culture of *Sporotrichum hummelii* (3 weeks old) and B2 (6 weeks old) (C) Cont. of *S. Tenckis* in hanging drop culture

Signs and symptoms The incubation period is 3 weeks to three months. The primary lesion may appear on the hand, arm or foot where the fungus is introduced through a wound. The lesion starts as a hard inelastic non-adherent movable nodule which undergoes softening, necrosis and ulceration resulting in a gummatous ulcer often called sporotrichotic chancre. Six clinical types have been described by Beurmann and Gougerot; namely (1) lymphatic (2) disseminated (3) epidermal (4) mucosal (5) skeletal and (6) visceral according to the site involved. From the site of primary infection the fungus spreads along the subcutaneous lymphatics. The lymphatics draining the area show subcutaneous nodules along the lymphatic vessels which become cord like. These secondary nodules soften and form abscesses some of which burst forming indolent gummatous ulcers (Pl. XXXV). This type of infection is common in U.S.A. Disseminated type is commonly seen in France and suggestive of haematogenous spread of the disease. No primary lesion is evident in this type and the onset is insidious. The first symptom noted is the presence of subcutaneous nodules scattered all over the body. These nodules contain viscid pus and rarely ulcerate. In the spreading type the infection may be insidious but multiple subcutaneous nodules burst and form tuberculoid syphylid or ecthymiform lesions. Mucosal lesions may be primary or secondary in dissemination through haematogenous route producing lesions also in viscera and brains. In the epidermal type there is extensive involvement of the skin with various types of lesions namely nodules either ulcerative or non ulcerative infiltrating plaques verrucose or papillomatous lesions.

Histopathology Lesions show only a non specific chronic inflammatory process similar to those in tuberculosis and syphilis. The granuloma shows infiltration with lymphocytes, plasma cells, giant cells and fibrosis. The method is therefore not useful for diagnosis. Fusiform bodies are usually absent in the section but may be seen in smears of pus.



Fig. 39.—Conidia of *S. schenckii* in culture

Miscology The fungus is dimorphic namely (a) tissue phase showing fusiform bodies and (b) filamentous form in culture. It is difficult to demonstrate the fusiform bodies in the pus. They are 1.5 by 4 μ and present in the mononuclear or polymorphonuclear leucocytes.

Culture The organism is better demonstrated by culture. The material is usually obtained from a nodule by incising it and tubes are inoculated and incubated at room temperature and in the incubator. Both blood agar and Sabouraud's agar should be used for culture. The primary culture appears in Sabouraud's glucose agar within 4 to 7 days but it may be delayed upto 10 days. It is at first creamy

and light brown in colour with an uneven leathery corrugated surface. With the age of the culture the colour becomes dark brown and the growth becomes wrinkled in the centre with radiating furrows. Excrescences appear in the centre of old cultures (Pl XXXV). Subcultures on cystine agar at 37°C show soft, yeast-like fusiform bodies.

Microculture. Branching mycelia are seen extending on all sides with terminal and lateral conidiophores bearing conidia. Conidia are pyriform varying from 4 to 8 in number and may form a rosette in some cases (Pl XXXV and Fig 39).

Biochemical reactions. Most of the strains liquefy gelatin. They show variable sugar reactions with formation of acid.

Animal inoculation. Most of the laboratory animals, specially white rats and mice, are susceptible and the intraperitoneal inoculations in rats produce acute inflammation of the testes.

Immunological reaction. Agglutinin has been demonstrated in patients and in experimental sporotrichosis. Spore suspensions are agglutinated in 1 in 600 dilution of patient's serum but sera of patients suffering from actinomycosis may agglutinate spores of sporotrichum (Widal 1910). Moreover the agglutination test is difficult as the spores have a tendency to spontaneous agglutination and normal sera may also agglutinate the spores in a variable titre. Intracutaneous test with an extract of sporotrichum is positive in infected subjects and a negative result excludes the infection. But occasionally false positive reactions are seen.

Diagnosis. Fusiform bodies found in experimental animals are rarely found in materials from human lesions. Positive culture from the pus of an unruptured abscess or biopsy material is the best method of diagnosis. The cultures are taken in blood agar and Sabouraud's glucose agar and incubated at room temperature and at 37°C. *S. schenckii* is identified by the characteristic colony with typical spore formation.

Treatment. Potassium iodide orally for a long period cures the disease. The drug should be continued for one or two months after the patient is apparently cured. Iodide-resistant cases may respond to sulphonamide therapy.

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CHAPTER XI

CLASS ASCOMYCETES

ALLESCHERIA BOYDII

(The asexual phase being *Monosporium apiospermum*)

Synonyms : *Monosporium apiospermum* Saccardo 1911 *Scedosporium apiospermum* Saccardo 1914 *Glenospora elapieri* Catanei 1927 *Indiella americana* Delamare and Gatti 1929

Definition *Allescheria boydii* is the perfect stage of *Monosporium apiospermum* causing mycetoma in U S A characterised by formation of white or light yellow granules constituted by wide septate hyphae in the tissue or exudate draining from the sinuses

History Although there are several previous records of mycetoma with white granules in U S A the first case to show a positive culture was one reported by Boyd and Crutchfield (1921) In the culture of the fungus they found conidial stage and ascospores The fungus was called *Allescheria boydii* Shear (1922) Emmons (1944) demonstrated that *A boydii* was the perfect stage of *Monosporium apiospermum* isolated from cases of maduromycosis in U S A

Symptoms The fungus causes mycetoma of the foot in U S A with multiple discharging sinuses The granules are white to yellowish white in colour It may cause bony destruction of metatarsals or phalanges of the foot like those caused by *Nocardia* group in general but the latter produces bone destruction more than *A boydii* or other types of infecting fungi

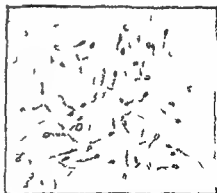


Fig 40—*A boydii* left—giant colony right—conidia in occult

Histopathology The lesions are like those of maduromycosis and formation of abscesses and sinuses is the prominent feature The granules are surrounded by polymorphonuclear neutrophils plasma cells lymphocytes eosinophils and macrophages Giant cells are often present The granules are spherical or lobulated and composed of thick septate hyphae with chlamydospores

Culture (Fig 40) *M. apiospermum* can be cultivated in ordinary media. In Sabouraud's glucose agar the colony develops rapidly as a white downy aerial growth which later becomes greyish to buff in colour. A black pigment is often seen on the reverse side of the growth.

Microculture (Fig 40) Microscopically the organism shows mycelia having single ovoid to clavate conidia with truncated base held at the end of conidiophores of various lengths. Corremia are sometimes seen with conidia at the end of conidiophores. Pleurogenous spores are also seen in certain strains.

In the ascomycetes phase of *A. boydii* the sexual spore is characterised by a cleistothecia (closed perithecia without ostiole) 50 to 200 μ in diameter and thin walled. They contain a brownish structure containing subglobose asci in each of which 8 ascospores 4 to 4.5 μ by 7 to 7.5 in size are present. As monosporous stage is the imperfect stage of *A. boydii* it is better to call the fungus *A. boydii*.

Animal inoculation Laboratory animals are not susceptible. Injection of a pure culture into the knee joints or feet of animals may produce only temporary lesions.

Diagnosis Granules that escape through the sinuses are characteristic. They are white or yellowish white in colour. Under the microscope mycelia are 2 to 4 μ in diameter and show chlamydospores at the periphery. After washing several times in sterilised normal saline the granules can be broken into small pieces and cultivated on Sabouraud's or beef infusion glucose agar and incubated at room temperature. Cultures should be kept for sufficient time for the growth which may be delayed.

Treatment Sulphonimides or antibiotics have not proved to be successful and the infected limb is to be amputated.

ASPERGILLUS

General characters The genus is characterised by formation of conidia borne on conidiophores which are unbranched non-septate erect aerial hyphae arising from foot cells growing on the substrate. Conidiophores form swollen vesicles at the tip which bear small flask-shaped sterigmata. Conidia are formed by a process of abstriction from the tip of the sterigma forming an unbranched chain. The proximal conidium is the youngest and the distal one is the oldest. The conidia are often prickly in appearance and disperse easily with the slightest disturbance (Fig 41).

Some *Aspergillus* show formation of loose perithecia which contain asci and ascospores and are called *Eurotium* (under Ascomycetes), whereas *Aspergillus* is classified by many under *Conidiophores* (Fungi Imperfecti).

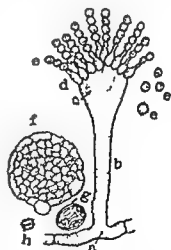


Fig 41—*A. penicillatus* (1) Foot cell (2) Conidia (3) Sterigmata (4) Conidium (5) Perithecia (6) Asci (7) Ascospores

Lesions *Otomycosis* is caused by *A. niger* or *A. flavus* and is likely to be secondary to bacterial infection of the ear caused by *Pseudomonas aeruginosa* and the fungus exists as a saprophyte in the macerated tissue or ear wax. It may cause irritation or unbearable pain in the ear. *Mastoidomycosis* is caused by *A. fumigatus* which has been isolated from the lesion. It is characterised by formation of black granules. It may produce a condition of bronchitis but it often occurs as a secondary invader in carcinoma of the lung, bronchiectasis or tuberculosis. Primary infection of the lung may simulate tuberculosis with chronic cough and mucopurulent discharge often containing blood. X-ray examination may show nodular or diffuse lesions resembling patchy bronchopneumonia, abscess of the lung or cavitation.

Diagnosis The organism is ubiquitous in its distribution and it is therefore difficult to ascribe the pathogenicity to a strain isolated from any natural orifice of the body or from sputum of the patient with pulmonary infection. Usually mycelial fragments are seen but conidiophores may be seen on rare occasions. Conidiophores are diagnostic but when mycelial fragments are seen the growth of *Aspergillus* must be obtained repeatedly from the material by culturing the material on Sabouraud's glucose agar.

PENICILLIUM

Penicillium is ubiquitous in distribution and often seen in rotten or decaying organic matters and it is one of the commonest food moulds often seen in fruits. They often contaminate laboratory culture media.

The fruit bearing organ is a conidiophore, an aerial hypha bearing conidia and forming finger-like branches resembling a hair pencil; hence the name. The conidiophore shows primary verticillate branches which on further subdivision form flask-shaped sterigmata abstricting at their tips chains of conidia (Fig. 42). Owing to its universal distribution in the laboratory it is difficult to ascribe the pathogenicity of any of these organisms isolated from the lesion. Different species of *penicillium* have been isolated from otomycosis and pulmonary abscesses. Penicillin is produced by *P. notatum*.

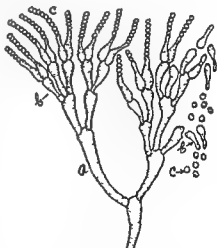


Fig. 42—*Penicillium* (a) bearing a long conidiophore (b) sterigmata (c) conidia

CHAPTER XII

CLASS PHYCOMYCETES

Order Mucorales Family Mucoraceae

Organisms of the family Mucoraceae are commonly known as bread moulds and are generally found in manured soil including horse dung heap, fruits and starchy food stuffs. The non-septate vegetative mycelia produce globose sporangia borne on sporangiophores which form columella at the end. They reproduce sexually by formation of zygospores. They are differentiated from fungi imperfecti by their coarse non-septate mycelia and loose aerial hyphae. The following genera are commonly encountered.

Mucor They show abundant floccose aerial mycelia at first white and then dark gray. Rhizoids are absent. Sporangiophores are simple or branched and arise from any part of the thallus. Spores are smooth and regular, round, oval or pear-shaped. Sporangia are single, multisporous and borne on the conidiophores forming columella, round or cylindrical in shape (Fig. 43). Common species—*M. mucedo*.

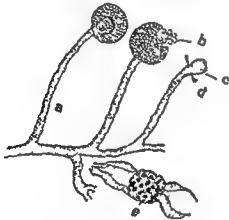


Fig. 43—*Mucor mucedo* (a) Sporangiophore
(b) Sporangium (c) Columella
(d) Apophysis (e) Zygospore



Fig. 44—*Rhizopus* (a) Rhizoid
(b) Sporangiophore (c) Sporangium
(d) Zygospore

Rhizopus They form dense colonies. Mycelia are at first white and then dark gray. They spread over the substrate by stolons or runners which connect a group of unbranched sporangiophores held by a tuft of root-like hyphae or rhizoids. Unlike *Mucor*, after dehiscence of the spores, columella are mushroom-like and rest on a cup-shaped expansion called apophysis. Spores are angular (Fig. 44). Common species are *R. niger*, *R. parasiticus*.

Ichthyemia In this genus mycelia are non-ramified with or without rhizoids. Peduncle supporting the sporangium terminates in a structure encircling the base of the columella. Species: *L. corymbifera* (*Mucor corymbifer*).

Rhizomucor These are mucor-like but rhizoids are present and columella are ovoid in shape.

Mucormycosis Note: *M. corymbifer* has been isolated from mycosis of the nose but its pathogenicity has not been definitely established. *Tungui*, *Rhizopus*.

niger has been isolated from black tongue. **EAR** Various species of *Mucor* have been incriminated for causing otomycosis. **NAILS** Species of *Mucor* have been isolated from cases of paronychia in orange workers.

LUNGS AND MENINGES Several cases of primary pulmonary mucormycosis with metastatic abscesses in various organs of the body before death have been recorded. Meningeal infection and abscesses of the brain caused by species of *Mucor* have been recently reported.

RHINOSPORIDIOSIS

Definition It is a chronic fungus infection caused by *Rhinosporidium seeberi* chiefly affecting the mucous membrane of the nose characterised by a polypoid growth. Cheek uvula lachrymal sac conjunctiva ear and mucous membranes of other parts of the body may be affected with formation of polypoid growths.

History Seeber (1900) reported two cases of nasal polyp caused by a protozoal infection. The organism was called *Coccidium seeberi* by Wernicke (1903) belonging to sporozoa. The third case was reported by O'Keefe (1903) in India and the organism was called *Rhinosporidium O'Keefe* by Minchin and Fantham (1905). Seeber (1912) found this to be identical with his organism and called the organism *Rhinosporidium seeberi*. Since then more cases were published from various parts of the world. The organism was thought to be a protozoa until Ashworth (1932) demonstrated the morphological similarity of the fungus with Chytridiales (Phycomycetes). The organism has not been cultivated in vitro and the taxonomic position of *R. seeberi* still remains doubtful.

Geographical distribution The disease is endemic in many parts of India and Ceylon. It has been reported from Mexico Argentina and other parts of South America U.S.A. Malay States Persia Italy Philippines and United Kingdom.

Etiology Infection may occur at any age but is commonly seen in children and young adults. Males are more often infected than females and divers are frequently infected. Those who dive swim or wash their face in stagnant water are usually infected. Karunaratne (1939) considered its transmission to be due to the custom of cleaning the nose in a common basin before entering the temple.

Source of infection The organism has not been cultured or transmitted experimentally to man or animals. The disease occurs spontaneously in horses cows and mules. It has been suggested that it is primarily a disease of fish and man and animals contract the disease accidentally from infected water.

Symptoms In rhinosporidiosis polypoid or papillomatous growth occurs on the nasal mucosa either sessile or pedunculated. They vary in colour from a pale pink to purplish red. Multiple minute white granules are seen on the surface of the new growth representing sporangia. Lesions may be seen in the eye and may affect the bulbar or palpebral conjunctiva eye lids producing papillomatous growths giving a foreign body sensation. Penile lesions appear as cauliflower like growths resembling venereal warts. There is no subjective symptom except that it causes inconvenience due to the growth.

Histopathology The polyp consists of a vascular myxomatous connective tissue and contains sporangia (Fig 46) with empty chitinous shells which may produce granulomas with foreign body giant cells sometimes with small abscesses.

Mycology The fungus is characterised by formation of sporangia 0.25 to 3 mm with a pore. It contains a large number of spherical or oval spores 6 to 7 μ in diameter with chitinous walls (Fig 46). They have vacuolated cytoplasm and a vesicular nucleus with a karyosome. Spores escape by rupture of the sporangium at the pore and are carried through the connective tissue to the lymphatic channels. The culture is not yet successful.

Animal inoculation Experimental inoculations in laboratory animals are not successful.

Prognosis The disease is rarely fatal although lesions may sometimes cause mechanical obstruction or deformities by their growths.

Treatment Surgical removal of the polyp should be done with a wire snare. Intravenous administration of antimony has been recommended and pentavalent antimony compounds intravenously upto a total dose of 2 to 4 gm may be helpful as an adjunct to surgical measures.

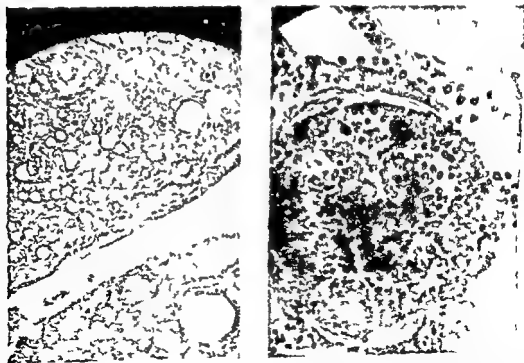


Fig 46—Left—Section of a nasal polyp showing various stages of development of sporangia $\times 80$ Right—Escape of spores by rupture of a sporangium through the pore $\times 430$

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APPENDIX A

SOLVENTS AND STAINS FOR DIRECT EXAMINATION

Sodium sulphide solution : To prepare a saturated solution put a few drops of distilled water to a crystal of Merck sodium sulphide. Take a volume of this solution and an equal volume of rectified spirit or 90 per cent alcohol. On mixing turbidity appears. Add a few drops of distilled water till the turbidity disappears. The solution is then filtered with a filter paper. The filtrate is obtained when a freshly prepared solution is used. But for all practical purpose this solution can be used in a drop bottle for one month without any deterioration.

Potassium or sodium hydroxide solution : A 10 per cent solution of potassium or sodium hydroxide solution is used as a keratolytic agent. For nails use a 40 per cent solution.

Sodium sulphide solution has been found to be the best of all the keratolytic agents and in the collection of the author this is better than potassium or sodium hydroxide solutions.

McGuire's stain

Iodine tincture 1 gm
Alcohol 1 cc
Acetic acid 4 cc
Water 100 cc

Carbol thionin

Thionin 1 gm
Phenol crystal 1 gm
Alcohol 30 cc
Water 100 cc

Borax methylene blue

Methylene blue 1 gm
Borax 5 gm
Water 100 cc

Thin scales like those of tinea versicolor erythrasma pityriasis capitis may be placed in a slide and treated with a few drops of McGuire's stain or carbol thionin for minutes. The stain is then mopped off with a piece of filter paper and a coverslip preparation is made with a drop of glycerine which gives a differentiating effect on the staining of the fungus. For scale of pityriasis capitis the scales are treated with ether or acetone for removal of fat so that they are treated with the stain. Borax or any other pyrolytic compound methylene blue stain may give a satisfactory result in this case.

Amman's Lactophenol (Modified)

Phenyletylate	0 gm	Lactacid (lyup)	10 gm
Glycerin	0 gm	Cotton blue	10 gm
Water	0 cc		

All the ingredients except cotton blue are dissolved with gentle heating. Cotton blue is added last and it dissolves in no time. Lactacid can be substituted for cotton blue with an equally good result and it gives a blue colour instead of a blue one. The stain is extremely useful for the culture mount. For the staining of sporangia, ulcers, etc. added with water for a trace of agar. The culture is then mounted in Amman's lactophenol in a cover slip preparation. Excess of the stain is removed by soaking with filter paper and the preparation is then allowed to dry on a glass slide.

COLLECTION OF MATERIAL

(For ringworm, tinea, varicella, etc.)

Equipment : (1) Forceps, slides wrapped in craft paper and sterilised in a lotus oven. (2) Ether and alcohol in a tumbler in equal volume. (3) Swab of cotton wool. (4) Paget's knife. (5) Epilation forceps. (6) Spirit lamp, etc.

The following points are recorded in the register namely—name, age, sex, date of collection, duration of illness, site from which the material is collected, in a diagnosis, result of direct examination, culture report and remarks.

Sampling from the glabrous skin : This is done for the examination of the scale and the culture. The patient to be scraped is cleaned and after dried with a swab of ether and alcohol.

mixture. The Paget's knife is dipped in ether alcohol mixture and passed through the flame of a burner or spirit lamp and thus the excess of spirit is burnt. The sterilized knife is used when cool. The scraping is usually done from the margin of the lesion without injuring the skin surface. This is done by scraping with the blade kept flat on the skin and not in an angular fashion. The slide should be held in such a way that scales obtained by scraping may be received directly on the slides. When sufficient material is collected the second slide is put on the first one on which the material was received. The slides are wrapped back in the craft paper. The serial number in the register is put down on the cover.

Collection of material from pompholyx. Boiled sago grain like blisters on the palm of the hand (Cheiropompholyx) or sole of the foot (Podopompholyx) are due to ringworm infection or id reaction. In the former case ringworm fungus is present and the skin from the blister shows ringworm fungi but in the latter instance no pathogenic organism can be detected. For collection of material from blisters a pair of scissors is similarly sterilized. The point of the scissors is introduced into the blister at its edge and the skin over the blister is cut at the periphery in a circular fashion holding the margin of the skin with a pair of dissecting forceps. The cut portion of the skin is received in between a pair of sterilized slides. Skin from several blisters may be collected in this way for direct examination and culture.

Scraping from the nail. Nails are liable to be attacked by ringworm fungi. The affected nail becomes opaque brittle and hypertrophied. Nails may also be affected by fungus. Collection is made by shaving the nail bed with a Paget's knife or with a hard safety razor blade. The material is received directly on the slide. Other procedures are the same as in the scraping of the glabrous skin.

Collection of infected hair. In ringworm of the scalp or beard area infected hairs are collected for direct examination and culture. This is conveniently done with the help of a pair of epilator forceps. It should be emphasized that stumps of infected hair are small and the epilator in such case is not at all useful. The fact should be borne in mind that if the epilator is useful it would be presumed that the epilated hair is already sterile and is just lying in the widely gaping hair follicles in cases of kerion of the scalp and trichomycosis.

CULTURE MEDIA

Sabouraud's proof medium

Malasse's dextrose (Chanut)	40 gm	Leptone (geotrichum) (100 mg)	10 g
Agar 15 gr	3 gr	Distilled water	1000 ml
		pH 5	

For the study of yeast colonies the above formula is usually followed but a slight difference in the composition of the medium or even the brand of the ingredients may alter the character of the growth of the yeast colony. In the tropics the quantity of agar should be increased.

In Italy Pollacci and in Germany Himdtzschke are used as a substitute for Sabouraud's medium. In America Weisman and Byring have demonstrated how dextrose is essential for the growth of *Trichophyton*. It was also demonstrated by Weisman and McMillan that crude American dextrose is not a suitable substitute for crude maltose de Chantrel in the original Sabouraud's medium. It was also found that technical dextrose gives equally good results.

Weidman's modification of Sabouraud's medium

Agar (granular)	18 gm	Leptone (Trichophyton)	10 gm
Dextrose (American crude)	40 gm	Water	1000 ml
		pH 6	

Preparation of Sabouraud's proof medium. Mix the ingredients in a flask and add the mixture to stand for half an hour. Then place the flask in the autoclave. Increase the temperature of the autoclave till a continuous jet of steam is coming out of the stopcock. Then stop the cock and the flask is cooled and the yeast is added to the medium. The flask is then placed in a water bath at 37°C.

half an hour. The gas is then turned off and when the steam has fully escaped the autoclave is opened. The solution is then filtered in a hot chamber and the pH is determined at 62 in a Hellige or Lovibond comparator. The medium is now distributed in test tubes and flasks (8 cc in a 16 l y test tubes or 15 cc Erlenmeyer flask to give a depth of about 1.5 cm). The tubes are plugged with yellow cotton wool (the colour indicate Sabouraud's proof medium). Flasks and tubes are then autoclaved. For this temperature of the autoclave is raised to 15 minutes when the gas is turned off. The pressure gradually falls to nil. The steam is allowed to escape and the autoclave is then opened. The tubes are then slanted on a slightly inclining plane and allowed to cool slowly. The flask is also allowed to cool without disturbance. The medium set when cool. The tubes are used for routine work of isolation and subculture and the Erlenmeyer flasks are used for culture of giant colonies.

C: As the reaction of the Sabouraud's medium is acidic too much or prolonged heating hydrolyse the sugar and prevents gel formation of agar. The processing should be carried right through without allowing the medium to solidify at any stage before the slope is made. Preparation of slopes by melting the stock solid medium should always be avoided as it has been found to be less effective to gel formation and to the growth of the fungus. Sabouraud's medium with gentian violet. This consists of Sabouraud's proof medium containing gentian violet in a proportion of 1:400,000 of the medium. For this one percent solution of gentian violet: kept as a stock solution and 0.5 cc of this solution is added to one litre of Sabouraud's proof medium. This medium has been found to be very useful in preventing the growth of contaminating organisms in culturing materials like nails or claws without interfering with the growth but the growth is delayed in this medium and is with five to seven days.

Malt extract agar medium (Difco) for culture of P. I (Moore's method)

Malto (Technical Difco)	1.75 gm	Ammonium chloride	0.75 gm
Malt extract (Difco)	1.00 gm	Bacto peptone (Difco)	1.00 gm
Dextrin (Difco)	5 gm	Bacto agar (Difco)	15.00 gm
Glycerine	0.7 gm	Water	1000 cc
Dipotassium phosphate	1.00 gm	pH 4.8	

Petroff's medium with gentian violet

100 gm of ground beef is infused in 1000 cc of 15 percent solution of glycerine in water in a refrigerator. Next morning squeeze the meat in a sterile press and collect aseptically in a sterile beaker.

Mix one part of meat juice and two parts of egg by volume. For this eggs are washed in soap and water and kept immersed in 70 percent alcohol for 10 minutes. Break the eggs in a sterile beaker and pass the mixture through sterile gauze.

To the mixture of infusion and egg add one percent alcohol solution of gentian violet to make a final dilution of 1:10,000 and mix the ingredients thoroughly. The mixture is distributed in a wrapped test tubes or bottles and stored by inspissation for three to five days.

Panaja's modification of Petroff's medium for culture of P. etc

Meat infusion in 15 percent glycerinated water } equal parts

Content of one egg

Gentian violet—0.004 percent slope added in pus to Meat infusion and egg is essential for the growth of P. etc

Blood agar and nutrient agar These are prepared as a routine bacteriological compact.

Corn meal agar : Corn meal 40 gm, Agar 20 gm and water 1000 cc.

Mix corn meal and water and tam in hot steriliser for about one hour. Filter through gauze and make up the volume. Add agar and mix in the autoclave (5 lbs pressure for one hour). Filter through into tubes and let lie in autoclave at 15 lbs pressure for 15 minutes.

N. B. C. nidulans is cultured in this medium to slow production of chlamydo spores.

Chlamydospore agar (Nickerson and Mankowski) This consists a basal medium to which purified starch is added. The basal medium consists of ammonium sulphate—1 gm potassium dihydrogen phosphate—1 gm biotin— μ Bacto agar—15 gm tryptic blue—0.1 gm and distilled water to make 1000 cc. Lunsford polysaccharide is sterilised separately by autoclaving or Tyndallization and added aseptically to sterilised basal medium in the proportion of 1 gm per 100 cc. Slit cultures are prepared in this medium by taking inocula from young cultures grown on glucose-yeast agar. Heavy inocula are put. The cultures are incubated at 25°C.

Purification of soluble starch Add 5 to 7 gm of commercial soluble starch to 100 cc of boiling water. When the starch is dispersed and clear liquid is still hot pour into an equal volume of saturated ammonium sulphate solution (60 gm of ammonium sulphate to 100 cc of the solution at 25°C). After standing for 24 hours the voluminous amorphous precipitate was filtered with suction washed with cold distilled water until the filtrate gave a negative test with Neill's reagent and then dried in vacuo. The starch solution should not contain any reducing sugar as determined by Fehling's reagent. With a dextran test a characteristic starch or starch like reaction. Some soluble starch sample gives a purple colour with iodine indicating a high content of dextrin.

Selective medium for isolation of pathogenic fungi In this medium cyclodextrin dextran agar is distributed in 100 cc quantities into each of the Erlenmeyer flasks and sterilised in an autoclave as usual. The flasks are then allowed to cool to 4°C and after bath and antibiotics to give the final concentrations of Cycloheximide (Actidione technical grade) penicillin and streptomycin are then added.

Stock Cycloheximide Solution Put 5 mg crystalline cycloheximide in a 50 cc volumetric flask and add 5–10 cc of acetone to dissolve it. Make up the volume with distilled water, 15 of which have been adjusted to 6–14 sterilise by passing through Seitz filter and store at 5°C. This will remain stable at 5°C for 6–8 weeks. Add 10 cc of stock cycloheximide to 100 cc of the cooled medium at 4°C to obtain 0.5 mg per cc.

From stock solution of streptomycin (4000 units per cc) and penicillin (2000 units per cc) add 1 cc each to 100 cc of the cooled medium to give a final dilution of 40 and 20 units respectively. This medium has been used for isolation of *Coccidioides immitis* from mycelia from clinical materials or from soil and other extraneous source.

H B Actidione the trade name of cycloheximide is distributed by Upjohn Company, Kalamazoo Michigan.

RECIPE FOR A 1 LITER BACTO MEDIUM

(Ink, iron and Mito levels)	
Soluble starch	10 gm
Maltose	10 gm
Glucose	10 gm
Erythrose	10 gm
Agar agar	0 gm
Water	1000 ml
When or potato starch has been found to be better substitute than soluble starch	

RECIPE FOR A 1 LITER BACTO MEDIUM

Dipotassium phosphate	10 gm
Potassium chloride	0.5 gm
Magnesium sulphate	0.5 gm
Biotin	0.01 gm
Agar agar	0.01 gm
(Ink, iron and Mito levels)	

RECIPE FOR A 1 LITER BACTO MEDIUM (for culture of *Aspergillus*)

Soluble starch	10 gm
Dipotassium phosphate	0.5 gm
Potassium chloride	0.01 gm
Magnesium sulphate	0.01 gm
Biotin	0.01 gm
Agar agar	0.01 gm
Distilled water	1000 ml
pH to 4	

100 cc of the cooled medium to give a final dilution of 40 and 20 units respectively.

APPENDIX B

COMMON THERAPEUTIC RECIPES

LOTIONS

LOTIO ACETICUS

Acetic acid 1 gr
Aqua ad 10 fl oz

To be applied on a figure which should be kept wet with the lotion 3 or 4 times a day. It is useful in treating infection of the skin.

LOTIO ALUMINUM ACETATE

Aluminum acetate 100 gr
Water 1000 cc
To be applied 3 or 4 times a day

LOTIO CALAMIN

Calamine 10 gr
Zinc oxide 10 gr
Glycerine 10 m
Liquor 10 fl oz
To be applied 3 or 4 times a day

LOTIO CALAMIN

Calamine 10 gr
Zinc oxide 10 gr
Glycerine 10 m
Liquor 10 fl oz
To be applied 3 or 4 times a day

To be applied 3 or 4 times a day. It is useful in treating infection of the skin.

LOTIO FORMALIN ET ACETICUS

Formalin 1 cc
Glycerine 10 m
Aqua ad 10 fl oz

It is useful in treating infection of the skin and feet. To apply on at night.

LOTIO ZINC

Zinc 10 gr
Specter 10 m
Olive 10 m
Specter 10 m
Specter 10 m
Specter 10 m
Specter 10 m

To apply on the scalp twice daily. It is useful in treating infection of the scalp. It does not stain the hair of blondes in the case of the orange.

LOTIO CANTHARIDIN

Cantharidin 10 m
Alcohol 10 cc
Aqua ad 100 cc

It is useful in treating infection of the skin.

LOTIO IODASOLU PERMANENT

Potassium iodate 100 gr
Water 1000 cc

LOTIO FLUOR

Iodine 100
Water 100 oz

To apply in case of infection of the skin.

LOTIO MERCURY

Mercury 4 gr
Olive 10 m
Specter 30 m
Specter 1 m
Specter 1 m
Rosa 10 fl oz
To apply in case of infection of the skin.

LOTIO MERCURY ET ACETICUS

Hg 100 gr
Acetic acid 100 cc
To apply on the skin.

LOTIO SULPHUREUS ALBA

Sulphur 10 g
Potassium iodate 10 g
Glycerine 10 m
Water or rose 10 fl oz

LOTIO SULPHUREUS ALBA

Sulphur (Precipitated) 10 gr
Liquor 10 fl oz
Water or rose 10 fl oz

It is useful in treating infection of the skin. It is useful in treating infection of the skin. It is useful in treating infection of the skin.

LOTION TRIPLE DYE

Acridavine	0.1 gm
Brilliant green	0.25 gm
Gentian violet	0.25 gm
Water	100 cc

Useful in intertrigo monilia is athletas foot tinea barbae sycosis barbae etc

LINIMENTS

LINIMENT CALAMINE

Calamina preparata	30 gr
Zinc oxide	30 gr
Alcohol	3 gr
Oil amygdal	4 dr
Liq calcis ad	1 fl oz

To apply on the patch of allergy or eczema or chronic dermatitis irritabilis skin etc

LINIMENT CALAMINE WITH ICHTHYOL

Ichthyol	10 gr
Imitant calamine	1 fl oz

Effacacious in seborrheic dermatitis

LINIMENT CALAMINE WITH SULFUR

Sulfur (Precipitated)	10 gr
Imitant calamine	1 fl oz

PAINTS

RINGWORM PAINT

Resorcin	1 oz
Acid salicylic	1 oz
Alcohol	1 oz
Acid acetic	1 fl oz
Glycerine	1 oz
Ethylmercuric nitrate	gr
Tincture benzoin co	4 oz

To apply on the patches of ringworm of the body

FICIENT RESORCIN BENZOIN CO

Resorcin	60 gr
Tincture benzoin co	1 fl oz

To apply on the ringworm patches including interdigital ringworm

FICIENT FUCHSIN (ASTELLANS PAINT)

Fuchsin sat alcoholic sol	10 cc
Percent of phenol	100
Boric acid	1 gm
Acetic	1 cc
Resorcinol	1 gm

Carbol Fuchsin —

Bismarckian sat alcoholic sol	10 cc
Phenol	5 gm
Water	100 cc

Add boric acid to filter. Carb. Fuchsin after hours add 10 cc of acetone and 10 hours later add 10 gm of resorcinol keep in a dark stoppered bottle

Apply on athlete's foot & ringworm of the nail

FICIENT THYMOL CINNAMON OIL AND IODINE

Thymol	10 gr
Cinnamon oil	10 gr
Tincture iodine sat	1 fl oz

To apply on the ringworm of the scalp and beard etc

POWDERS

POLY SELLER ET AMERSON

Sulfur (precipitated)	
Camphor	
Zinc oxide	
Boric acid sat	10 gr
Poly amylum sat	1 oz

To be kept in a stoppered powder can To dust on the affected areas of seborrheic patches & ringworm infection

UNDER THE ATLAS PAPER

Zinc undecylenate	10 gr
Undecylenic acid	10 gr
Talcum powder	1 part

This should be used as a prophylactic in tinea cruris and interdigital ringworm

FICIENT POWDER

Zinc undecylenate	1 part
Undecylenic acid	1 part
Talcum powder	10 parts

To be kept in a stoppered powder can

FICIENT POWDER

Calcium hypochlorite	1 part
Zinc hypochlorite	1 part
Potassium	1 part

To apply locally on the skin of ringworm as a prophylactic powder

SHAMPOO

SPIRIT AETHER SOAP SHAMPOO

Sapo vir d	1 o
Aether	
Alcohol rectified pint each	1 o

To apply on the scalp by rubbing thoroughly and then to wash. This should be done twice weekly in scborrioca capiti and pruritus.

SPIRIT

SPIRIT HYPOCRIN ET HYPOCRIN

Hg perchloride	1 gr
Re ore n	1 gr
Spirit vinum rect	1 0 m
Water a l	1 fl o

To be applied 1/2 lb 1 t mm
loils et

UNGUENTUM (Ointment)

UNGUENTUM DITHIRACOLIS

Dithranol	n gr
Laraffin molle	10 gm

To apply on 1 t l of 1 lb 2 o n a l
1 s 1

The stre 6 l e n l o s s reas d f tol rat i
Its a t o n is l i k e t h a t o f e r y s e r o t b u t i t
s c 4 l m e s m o e c l e v e t h n i t

UNGUENTUM HYPOCRIN ET HYPOCRIN

Hydra g mmon	10 lb k
Hyd ou ointm nt a l	1

For 10 in m i e i g

UNGUENTUM WHITENLI

Acid benzoic	20 gr
Acid salicylic	10 gr
Oil coco	4 dr
Laraffin durum	4 dr

For paratibacofre by melting and then
add other ingredients

To apply on the ringworm of the groin
and other sensitive parts of the body

UNGUENTUM HYPOCRIN ET HYPOCRIN

Iodine (Crystal)	10 parts
Lanolin	100 part

To apply locally as antiseptic or counter
irritant

QUINOLIN CHLORIDE OINTMENT (Squibb)

Chloroquinol	10 parts
Hydroquinol	100 parts
Aromatic oil	100 parts
Vehicle (Inert)	100 part
To apply in cases of erythema	

UNGUENTUM ALUMINUM ET HYPOCRIN

3 parts of aluminum in water solution
1 lb

Iodine ointment B1

UNGUENTUM HYPOCRIN ET PROPIONATE

Sodium propionate	10 parts
Propionic acid	36 parts
Hydroquinol	100 parts
Zinc stearate	10 parts
Carnauba wax	10 parts

For prophylaxis of skin worm

HYPOCRIN OINTMENT

Zinc undecylenate	1 part
Undecylenic acid	3 parts
Water soluble	100 part

For prophylaxis of skin worm

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